FACTORS AFFECTING THE POPULATION DYNAMICS OF *THEILERIA PARVA* IN RHIPICEPHALID TICKS

By

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DECLARATION

This thesis is the result of original research conducted by myself, unless stated otherwise in the text or acknowledgements, under the supervision of Drs. Graham F. H. Medley, Alan S. Young and Brian D. Perry. All sources of information have been specifically acknowledged.

No part of this thesis has been submitted for a degree at any other university.

SUMMARY

A series of experiments were carried out to investigate some of the poorly understood aspects of the life cycle of *Theileria parva* in its rhipicephalid tick vectors. The first series of experiments established that nymphae infected as larvae develop lower levels of infection compared to adults infected as nymphae, while female ticks develop higher infections than males. It was shown that the period of development of sporoblasts into mature sporozoites took on average four days in the nymphal ticks compared to five days in the adult ticks. Infection levels developing in different tick instars or sexes appeared to be related to the number and position of type III salivary gland acini. The second series of experiments established that there were considerable differences in the vector competence of different stocks of *Rhipicephalus appendiculatus* and *R. zambeziensis* for the transmission of Muguga and Boleni stocks of *Theileria parva*. Finally the study established that survival of infected *R. appendiculatus* and the *T. parva* they harboured was longer under quasi-natural climatic conditions compared to all the laboratory conditions examined. Basically, infection levels in the ticks did not affect the duration of survival of the ticks, however, survival of the parasite appeared to be influenced by the intensity of infection in the tick as the parasites diminished more rapidly in ticks having high infections than in those having low infections. Nymphae and the parasites they harboured survived for shorter periods compared to the adult ticks and their infections. Data generated from these series of experiments will be used to develop quantitative models of *T. parva* dynamics in the tick vectors.

The relative importance of the factors influencing the levels of infection developing in the tick vector were analysed statistically by the logistic and Poisson regression. Factors found to play a significant role included tick instar or gender, tick stock, parasite stock, the ambient climatic conditions in which infected ticks survived and the day of tick repletion after infection of the bovine host. Individually, the bovine host or its piroplasm parasitaemia were found to be poor predictors of infection levels developing in the salivary glands of the tick vector. However, when piroplasm parasitaemia was included in a model lacking the days post-repletion variable, the bovine host factor became significant.

CHAPTER 1

INTRODUCTION

1.1 Terminology

The nomenclature used to describe the different theilerial parasite populations throughout this thesis conforms to that recommended by Irvin, Dobbelaere, Mwamachi, Minami, Spooner and Ocama (1983).

Isolate viable organisms isolated on a single occasion from a field sample in experimental hosts, culture systems or stabilate.

Stock all the populations derived from an isolate without any implication of homogeneity or characterization. This definition was also applied for tick populations.

Stabilate a sample of organisms preserved alive (usually in replicate) on a single occasion.

Line a laboratory derivative of a stock maintained under defined physical conditions, for example, as a culture of parasitized bovine lymphoid cells.

Parasite clone *Theileria* species line derived from a single parasite.

Cell clone *Theileria* species line derived from a single parasitized cell.

Strain a population of homogeneous organisms possessing a set of defined characters.

Terms used to describe levels of infection conform to those given by Büscher and Otim (1986).

Prevalence number of infected ticks per number of ticks examined.

Abundance number of infected acini per tick examined.

Mean intensity number of infected acini per *infected* tick.

A *batch* of ticks was defined as a group of ticks of the same stock that fed to repletion on an animal on the same day.

1.2 Theileriosis due to *Theileria parva*

1.2.1 The parasite

Theileriosis caused by *Theileria parva,* a haemoprotozoan parasite of the class Sporozoea, is a very important cattle disease in eastern, central and southern Africa. The parasite has coexisted, rarely causing mortality (Neitz, 1957), with the African buffalo *(Syncerus caffer),* an indigenous bovine of sub-Saharan Africa, long before cattle were introduced into the region. It is postulated that a sub-population of this

parasite may have become adapted to cattle introduced into eastern Africa to form the present day East Coast fever (ECF) parasite (Uilenberg, 1981; Grootenhuis, 1989).

Theileria parva manifests itself in cattle as classical ECF, Zimbabwean theileriosis (also known as January disease) and buffalo-derived Corridor disease. At one time, parasites causing these disease syndromes were accorded full species status, that is, *T. parva, T. bovis* and *T. lawrencei* respectively (Irvin, 1987). This was as a result of the distinctive clinical differences between the three diseases in cattle. *Theileria parva* and *T. bovis* are primarily transmitted between cattle, but the disease caused by the former generally produces a greater abundance of schizonts and piroplasms than the latter. *Theileria lawrencei* generally produces fewer schizonts and piroplasms in cattle than either *T. parva* or *T. bovis* but causes a much more acute disease with a higher mortality rate. In the late nineteen seventies, a trinomial nomenclature was introduced dividing *T. parva* into three subspecies: *T. parva parva* for the parasite causing classical ECF, *T. parva lawrencei* for the buffalo-derived parasite, and *T. parva bovis* for the parasite causing January disease in Zimbabwe (Uilenberg, 1976; Lawrence, 1979; Uilenberg, 1981). This nomenclature was introduced because of the apparent epidemiological distinction between cattle and buffalo-derived disease as well as to distinguish between the cattle-derived disease in Zimbabwe and that occurring to the north of Zimbabwe. The criteria used to distinguish these three alleged "subspecies" were based on subjective, vaguely defined epidemiological and clinical features as the parasites were indistinguishable morphologically and serologically (Uilenberg, 1981). This system of nomenclature did not last long however as there was no clear phylogenetic basis for this distinction. This confusion in nomenclature has been partly due to the use of non specific diagnostic techniques which are unable to distinguish the parasites. Apart from the confusion in nomenclature, a need for more reliable

characterization techniques for theilerial stocks are essential for other reasons. For example, an effective method of immunization against *T. parva* by infection and treatment (Radley, 1981) has not been fully exploited due to the perceived differences in parasite stocks.

Currently, serological and the powerful nucleic acid based diagnostic techniques have failed to distinguish cattle and buffalo-derived theilerial parasites from each other (Lohr & Ross, 1969; Burridge, Kimber & Young, 1973; Lawrence, 1979; Allsopp, Baylis, Allsopp, Cavalier-Smith, Bishop, Carrington, Sohanpal & Spooner, 1993). Thus, it is now generally accepted that *T. parva parva, T. parva bovis* and *T. parva lawrencei* are merely behavioural forms of the same species and not true subspecies (Anonymous, 1989). However, some workers suggest that there may be genetic differences between certain cattle and buffalo-derived *T. parva* isolates (Morzaria, Bishop, Nene, Spooner & Gobright, 1993). As Perry and Young (1993) aptly stated however, confusion over the nomenclature of cattle and buffalo-derived theilerial parasites has not abated either in terms of understanding earlier nomenclature decisions or of the determinants of the different epidemiological states of *T. parva* infections in the field. Due to this uncertainty, descriptive terms such as "cattle-derived", "January disease", "buffalo-derived or Corridor disease", and so on, will be used throughout in this thesis. A geographical name will also be included where necessary to show the origin of the *T. parva* isolate.

1.2.2 Tick vectors

The principal field vector of *T. parva* is the brown ear tick, *Rhipicephalus appendiculatus*. This is a three host ixodid tick that becomes infected by feeding on infected bovid hosts as larvae or nymphs and transmits the parasites in the next tick

instar. Transmission is always transstadial and transovarian transmission has not been demonstrated. Other known field vectors, where geographical distribution and *T. parva* occurrence is correlated, are *R. zambeziensis*, in the hotter, drier areas of central and southern Africa, and *R. duttoni* in Angola and Zaire (Lessard, L'Eplattenier, Norval, Kundert, Dolan, Croze, Walker, Irvin & Perry, 1990; Norval, Perry & Young, 1992a). The distribution of the vectors of *Theileria parva* is shown in Figure 1.1.

1.3 Distribution

Theileriosis due to *T. parva* is restricted to eastern, central and southern Africa. Clinical theileriosis and the presence of antibodies to *T. parva* have been reported in Kenya, Angola, Botswana, Burundi, Malawi, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Zaire, Zambia and Zimbabwe (Dallwitz, Young, Mahoney & Sutherst, 1986; Lessard *et al*, 1990). Its distribution is intimately correlated with that of its tick vectors and it has never established itself in areas where the tick vectors are absent (Barnett, 1977; Young, 1981). However, the parasite may be absent within areas of the tick vector distribution. This may be due to lack of infection in the ticks or lack of suitable conditions for parasite development and survival within the tick (Young, 1981; Walker, 1990). Furthermore, even in countries where the tick vectors are widely distributed, they are restricted by several factors to certain ecological zones. The most important of these factors are climate, vegetation and host availability.

Theileria parva has a restricted host range, infecting cattle, the African buffalo *(Syncerus caffer)* and waterbuck *(Kobus ellipsiprymnus* Ogilby 1833) (Stagg, Young, Leitch, Grootenhuis & Dolan, 1983). The epidemiology of *T. parva* is further influenced by the fact that indigenous cattle breeds (Zebu and Sanga) and types are

more resistant to *T. parva* infection (Young, Leitch & Newson, 1981; Moll, Lohding, Young & Leitch, 1986) and *R. appendiculatus* infestation (Rechav & Zeederberg, 1986; Norval, Sutherst, Kurki, Gibson & Kerr, 1988) than the exotic Taurine breeds and types. Further, there is evidence of varying susceptibility to the parasite and the tick vector even among the indigenous breeds and types of cattle.

Wild animals play an important role in the epidemiology of *T. parva.* Buffalo-derived *T. parva* may be present wherever the distribution of the African buffalo and the rhipicephalid tick vectors overlap (Uilenberg, 1981). The African buffaloes are particularly important as virtually all are carriers (most probably life-long), as was demonstrated in a survey undertaken in various areas of eastern Africa, where all those examined had *Theileria* piroplasms in their erythrocytes (Young, Brown, Burridge, Grootenhuis, Kanhai, Purnell & Stagg, 1978). This is a very important feature because many domestic cattle in Africa share grazing land with wild animals, including the African buffaloes (Young & Grootenhuis, 1985). Another important epidemiological feature is that carrier buffalo apart from producing ticks with relatively high infection rates and levels compared to those from carrier cattle (Young & Grootenhuis, 1985; Young, Leitch, Newson & Cunningham, 1986; Grootenhuis, Leitch, Stagg, Dolan & Young, 1987; Maritim, Young, Lesan, Ndungu, Mutugi & Stagg, 1992), are also most probably life long carriers.

Theileria parva infection levels established in ticks that feed on carrier cattle are normally very low compared to those established in ticks that feed on cattle undergoing acute infections (Young & Grootenhuis, 1985; Maritim, Young, Lesan, Ndungu, Mututgi & Stagg, 1989). However, carrier cattle play an important role in *T.*

parva transmission because in some endemic areas, almost 100% of adult cattle are carriers (Young *et al.,* 1986; Mutugi, Young, Kariuki, ole Tameno & Morzaria, 1991).

By defining the climatic requirements of *R. appendiculatus*, the climate matching model CLIMEX can be used to predict the favourability of any particular area for the tick. CLIMEX calculates an ecoclimatic index (EI), which describes the favourability of a location for the tick species on a scale of 0 to 100, from a growth index (GI) and hot, cold, wet and dry stress indices (Maywald & Sutherst, 1987; Lessard *et al.,* 1990). There is a very close correlation between the distribution of ecoclimatic index values and that recorded for *R. appendiculatus* in much of eastern Africa, especially in Kenya, Burundi, Rwanda and Uganda. There are some exceptions however, for example, in central Tanzania, where even though climatic suitability exists, the tick has not been recorded (Perry, Lessard, Norval, Kundert & Kruska, 1990a).

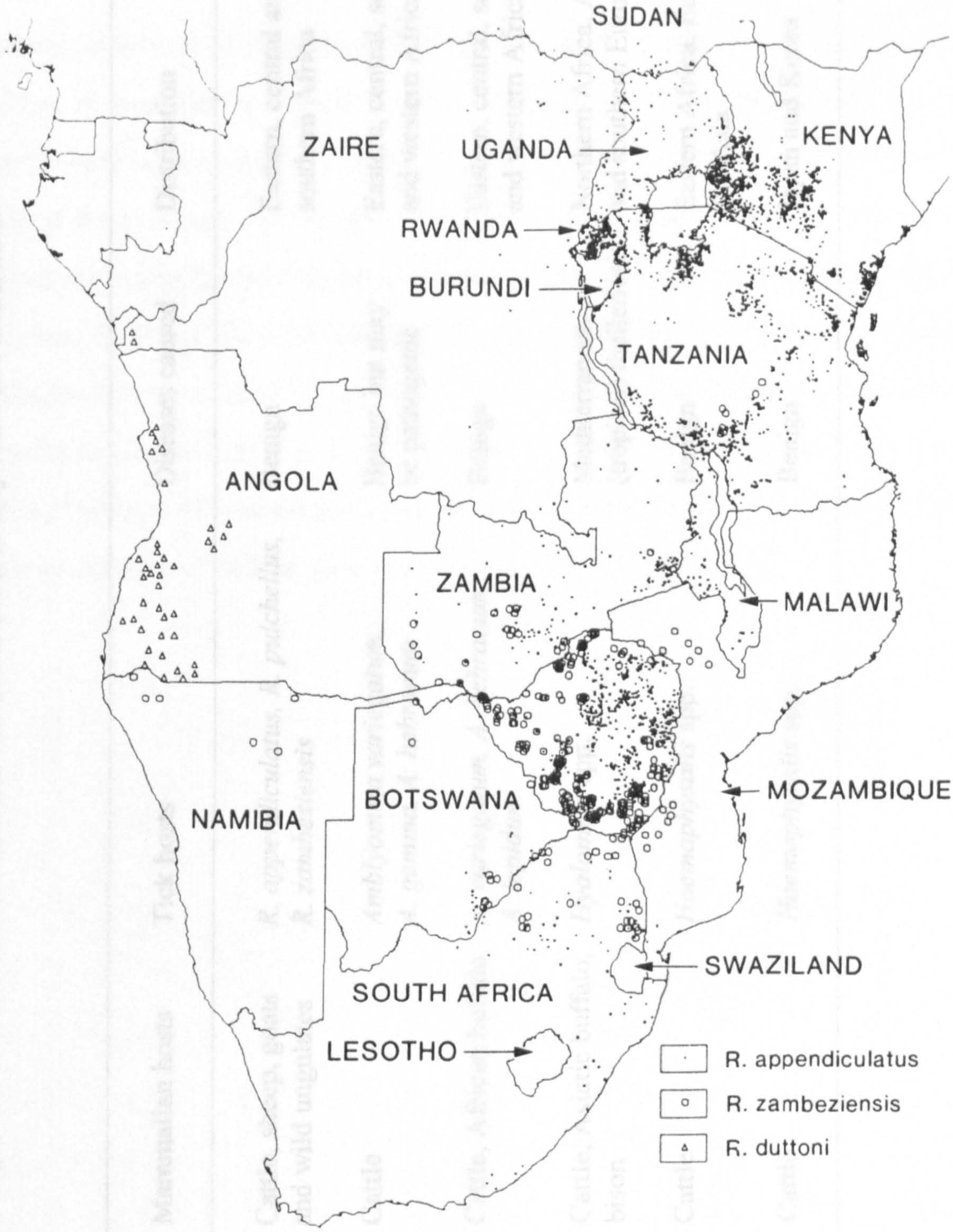


Figure 1.1. Map showing the distribution of *Rhipicephalus appendiculatus, Rhipicephalus zambeziensis* and *Rhipicephalus duttoni* in Africa (from Norval *et al.*, 1992a).

Table 1.1. Other *Theileria* species, their mammalian and field tick hosts, diseases they cause and distribution.

Theileria species	Mammalian hosts	Tick hosts	Diseases caused	Distribution
Theileria taurotragi	Cattle, sheep, goats and wild ungulates	*R. appendiculatus, R. pulchellus, R. zambeziensis*	Benign	Eastern, central and southern Africa
Theileria mutans	Cattle	*Amblyomma variegatum, A. gemma, A. hebraeum*	Benign but may be pathogenic	Eastern, central, southern and western Africa
Theileria velifera	Cattle, African buffalo	*A. variegatum, A. hebraeum, A. lepidum*	Benign	Eastern, central, southern and western Africa
Theileria annulata	Cattle, Asiatic buffalo, bison	*Hyalomma* spp.	Mediterranean (tropical) theileriosis	Northern Africa, Asia and southern Europe
Theileria buffeli	Cattle	*Haemaphysalis* spp.	Benign	Eastern Africa, Europe and Asia
Theileria sergenti	Cattle	*Haemaphysalis* spp.	Benign	Japan and Korea

Serological surveys of antibody prevalence to *T. parva* have also been carried out in a number of countries in the ECF region. The large scale of the surveys may not provide very accurate ECF distribution but they do give a fairly good indication of the scale of the problem. For example, in a national survey carried out in 36 districts of Kenya, excluding the four northern frontier districts of Turkana, Marsabit, Wajir and Mandera, 31.3% of the cattle tested for *T. parva* antibodies by the haemaglutination inhibition test were found to be positive (FAO, 1975). The distribution of antibody prevalence rates by district is shown in Figure 1.2. In another report on theileriosis in Kenya, Duffus (1977) stated that about 50% of cattle were in areas *R. appendiculatus*. Thus an outbreak of ECF in these areas could be quickly established.

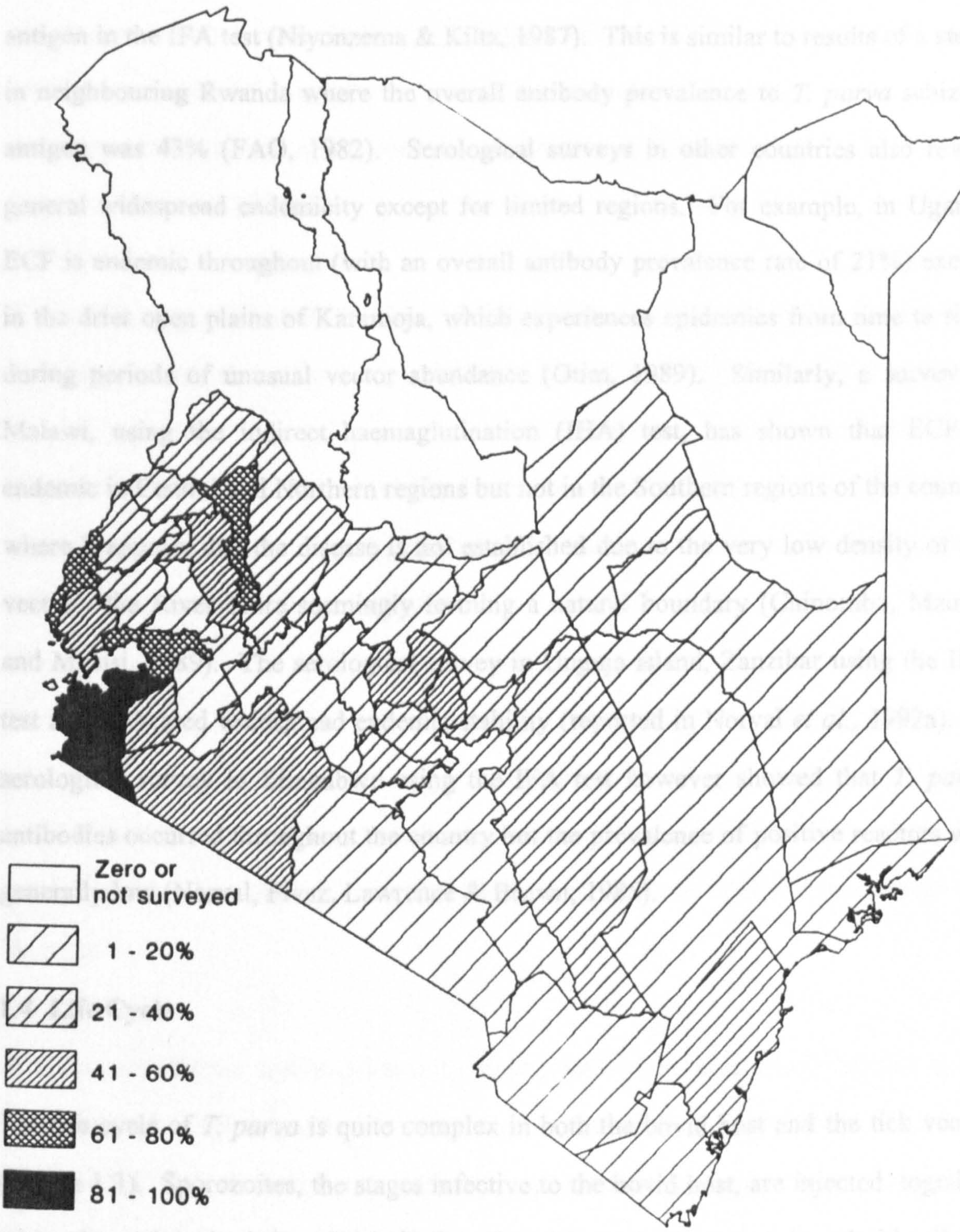


Figure 1.2. Map showing the distribution of prevalence rates of antibodies to *Theileria parva* IHA antigen in districts of Kenya surveyed by FAO (1975) (presented in Norval *et al.*, 1992a).

Similarly, a countrywide serological survey undertaken in 1980 in Burundi revealed that on average 63% of cattle had detectable antibodies against *T. parva* schizont antigen in the IFA test (Niyonzema & Kiltz, 1987). This is similar to results of a study in neighbouring Rwanda where the overall antibody prevalence to *T. parva* schizont antigen was 43% (FAO, 1982). Serological surveys in other countries also reveal general widespread endemicity except for limited regions. For example, in Uganda ECF is endemic throughout (with an overall antibody prevalence rate of 21%) except in the drier open plains of Karamoja, which experiences epidemics from time to time during periods of unusual vector abundance (Otim, 1989). Similarly, a survey in Malawi, using the indirect haemaglutination (IHA) test, has shown that ECF is endemic in Central and Northern regions but not in the Southern regions of the country where it appears that the disease is not established due to the very low density of the vector - the River Shire seemingly forming a natural boundary (Chinombo, Mzoma and Musisi, 1989). The serological survey in Unguja island, Zanzibar using the IFA test also indicated widespread endemic stability (reported in Norval *et al.,* 1992a). A serological survey in Zimbabwe using the IFA test however showed that *T. parva* antibodies occurred throughout the country but the prevalence of positive reactors was generally low (Norval, Fivaz, Lawrence & Brown, 1985).

1.4 Life Cycle

The life cycle of *T. parva* is quite complex in both the bovid host and the tick vector (Figure 1.3). Sporozoites, the stages infective to the bovid host, are injected together with saliva when the infected tick feeds and rapidly penetrate target lymphoid cells of the susceptible host through a defined sequence of events which are described in detail by Fawcett, Doxsey, Stagg and Young (1982a) and Shaw, Tilney and Musoke (1991).

Multiple infections are frequent and up to eight schizonts may develop in a single host cell but these cells eventually die out (Stagg, Dolan, Leitch & Young, 1981). As the parasite grows into a schizont, the host cell gets transformed into a lymphoblast which produces two infected daughter cells resulting in a clonal expansion with a ten-fold increase of infected cells every three days or so (Jarrett, Crighton & Pirie, 1969; Irvin, Ocama & Spooner, 1982).

It has been suggested that the term *"Theileria*-induced reversible transformation" is more appropriate than clonal expansion (ole-Moi Yoi, 1989) since the transformed lymphocytes revert to a quiescent state when treated early with antitheilerial drugs (Pinder, Kar, Mayor-Withey, Lundin & Roelants, 1981; ole-Moi Yoi, 1989). All lymphoid tissues of the body are infiltrated within 12 to 14 days after infection and schizonts start undergoing merogony.

The actual mechanisms of *T. parva* merogony, where numerous identical individual cells develop from a multinucleated syncytium are quite elaborate and are described in detail by Mehlhorn and Schein (1984) and Shaw and Tilney (1992). On completion of merogony, the host cell plasma membrane ruptures and mature merozoites are liberated.

Merozoites penetrate erythrocytes and develop into piroplasms which assume either comma or spherical shapes and lie free within the erythrocyte cytoplasm. Mehlhorn and Schein (1984) claim that only the comma shaped forms divide. However, limited division by schizogony in spherical forms to form the Maltese-cross tetrad forms has been demonstrated in *T. parva* by Conrad, Denham and Brown (1986) and Fawcett, Conrad, Grootenhuis and Morzaria (1987). Spherical forms are thought to be

precursors of gametes (Schein, Warnecke & Kirmse, 1977; Mehlhorn & Schein, 1984). Detailed descriptions of the ultrastructure of the piroplasms are given in Mehlhorn and Schein (1984), Conrad, Kelly and Brown (1985), Conrad *et al.* (1986) and Fawcett *et al.* (1987).

Piroplasms are the parasite stages infective to the tick vectors and a vast majority of them are rapidly destroyed in the gut lumen after ingestion of a blood meal (Walker, 1990). Thus in only 10% of any tick population do the parasites survive in large enough numbers to produce detectable sexual stages (Young, Shaw, Ochanda, Morzaria & Dolan, 1992). Soon after repletion, so called "strahlenkörper" or ray bodies, believed to be microgametes, form in the tick gut. These develop rapidly into short-lived, uninucleated spindle shaped structures (Young, Grootenhuis, Leitch & Schein, 1980). Within 48 hours post-repletion, relatively large spherical stages which appear neither to form protrusions nor to undergo division also develop from the ring form piroplasms. These forms are considered to be macrogametes. (Mehlhorn & Schein, 1984; Young *et al.*, 1980). Time taken to syngamy of gametes in the tick gut depends on the ambient temperature (Young & Leitch, 1981). The resultant spherical zygote enters a gut epithelial cell where it lies free within the host cell cytoplasm (Mehlhorn & Schein, 1984). The zygote then develops into a single, motile, club-shaped kinete. Even though the mature intracellular kinetes are mostly unicellular, nuclear division in *T. parva* kinetes starts so early that kinetes with up to four nuclei are often found inside the tick gut cells (Mehlhorn, Schein & Warnecke, 1978).

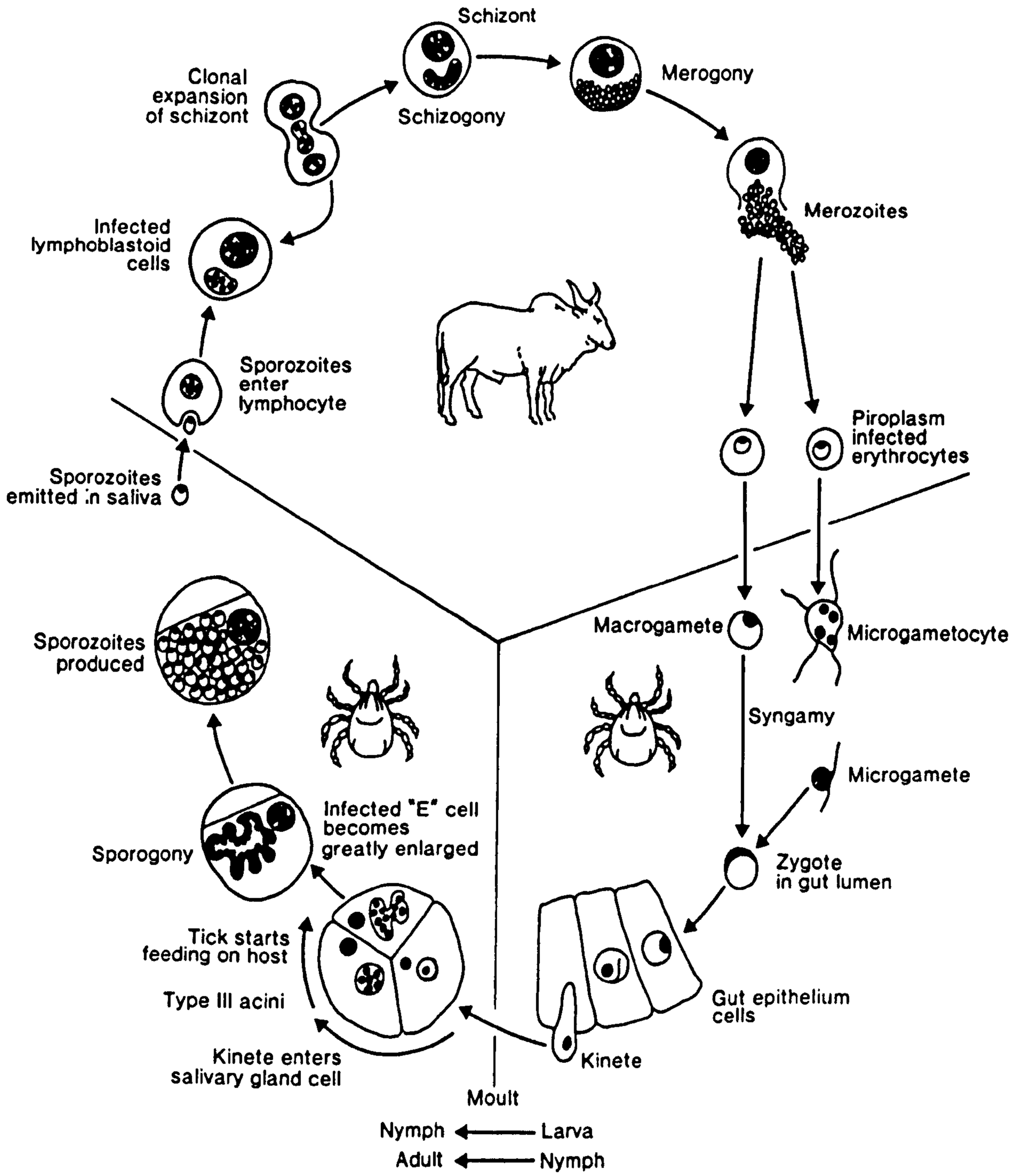


Figure 1.3. Life cycle of *Theileria parva* (from Norval *et al.*, 1992a).

Kinetes appear in the haemolymph during or immediately after tick moulting (Schein *et al.*, 1977; Young *et al.*, 1980; Young & Leitch, 1980; Mehlhorn & Schein, 1984) and it seems that they only enter the salivary glands after each moult.

Salivary gland infection by kinetes may be a chance event (Bell, 1980; Walker, 1990; Shaw & Young, 1994), however *T. parva* develops only in the *e* cell of the type III salivary gland acinus (Fawcett, Büscher & Doxsey 1982a; Binnigton, Young & Obenchain, 1983). After entry into the *e* cell, the parasite develops into a sporont which, as in all the other intracellular stages of the parasite's life cycle, is not surrounded by a parasitophorous vacuole (Fawcett, Doxsey & Buscher, 1982b; Mehlhorn & Schein, 1984; Fawcett, Young & Leitch, 1985).

A variable degree of parasite development occurs even in the unfed tick. The parasite develops into a multinucleated syncytium, called a sporoblast then appears to become dormant until tick feeding commences. However, it has been shown that exposure of ticks to high ambient temperatures of 28°C and above for several days can stimulate the completion of sporogony (Young, Leitch & Omwoyo, 1979; Young, Leitch & Mutugi, 1984; Ochanda, Young, Mutugi, Mumo & Omwoyo, 1988). When tick feeding commences, the parasite mass increases rapidly and the host cell nucleus and cytoplasm increase further in size. Ultrastructural studies have now shown that the sporoblast does not develop in a stepwise process but rather as a ramifying, multinucleate syncytium that gives rise to sporozoites in a terminal process of segmental fission (Fawcett *et al.* 1982a, 1982b, 1985). Development of the sporoblast into mature individual uninucleate sporozoites generally takes three to four days after tick feeding commences (Purnell, Brown, Cunningham, Burridge, Kirimi & Ledger, 1973; Young, Purnell, Kimber & Payne, 1975). On completion of sporogony, each

acinar cell in the adult tick salivary gland contains 30-50,000 sporozoites (Fawcett *et al.*, 1985).

1.5 Transmission dynamics of *Theileria parva*

Mathematical models are useful tools in studying the epidemiology of *T. parva* and other tick-borne diseases. They provide information on the relative importance of various factors that influence the interactions of host-parasite relationships. This information is useful in setting research agendas and in designing robust tick and tick-borne disease control strategies. While tick population dynamics models have received attention and some models developed, for example, the three-host tick model, T3HOST (Maywald, Dallwitz & Sutherst, 1980; Floyd, Maywald, & Sutherst, 1987) and ECFXPERT (Byrom, 1990), attempts at developing tick-borne disease transmission models are few. Gettinby and Byrom (1989) have developed an ECF model contained in the ECFXPERT model. Recently, Medley, Perry and Young (1993a) developed mathematical models that could be used to evaluate the effect of two control intervention models influencing the transmission of *T. parva* by *R. appendiculatus* to cattle.

Even though numerous factors influence directly or indirectly the epidemiology of *T. parva*, the degree to which they do so vary widely. One of the most important factors that influence the epidemiology of theileriosis is the population dynamics of the tick vectors. The population dynamics of the tick vectors themselves is dependent on climate, which affects the development, survival and behaviour of the tick. Central and southern African tick populations undergo a period of behavioural diapause in response to adverse weather conditions. While this results in seasonal transmission of

T. parva, its effect on parasite survival is yet to be determined. This is just one aspect of the numerous factors that the epidemiology of theileriosis is directly or indirectly dependent on. However a major problem is that while some factors, like tick infection rates, may be easily measurable, others are not. These factors are discussed in detail in Norval *et al.*(1992) and Shaw and Young (1994).

1.6 Economic impact of ticks and tick-borne diseases

Theileriosis caused by *T. parva* still remains one of the major constraints to the development and improvement of the livestock industry in all the eleven eastern, central and southern African countries where it occurs. Apart from direct mortality, other substantial losses include productivity losses in recovering or recovered cattle, loss of grazing land, control costs and extension services related to it and the financing of diagnosis, training and research. Particularly hard hit are the high yield Taurine, Taurine/Zebu and Taurine/Sanga improved cross breed cattle (Duffus, 1977; Callow, 1983; Morzaria, Irvin, Wathanga, D'Souza, Katende, Young, Scott & Gettinby, 1988; Mukhebi, 1992) where morbidity and mortality due to *T. parva* infections can approach 100% (Cunningham, 1977). Indigenous breeds subjected to intensive tick control, or moved from disease-free to endemic areas also die in large numbers (Ngulo, 1985; Barnett, 1977). Death of cattle under endemically stable conditions occur mostly in calves and may reach 50% of the total calf population (Moll, Lohding & Young, 1984; Moll *et al.,* 1986; Ngulo, 1985; Ngulube, Ellwood & Radley, 1985; Berkvens, Geysen & Lynen, 1989; Otim, 1989). Cattle in endemically unstable areas are even more vulnerable and mortality rates may be as high as 80 - 100% in calves (Julla, 1985). In 1989, using a computer spreadsheet model, Mukhebi, Perry and

Kruska (1992) estimated the total regional loss due to theileriosis at US$ 168 million per year, which included an estimated mortality of 1.1 million cattle.

Governments of the affected countries spend substantial amounts of money, mostly in the form of foreign exchange, mainly for the importation of chemotherapeutic drugs and acaricides for tick and tick-borne disease control. For example, in 1987, Kenya spent approximately US$ 10 million in the importation of acaricides and chemotherapeutic drugs for the control of theileriosis (Young, Groocock & Kariuki, 1988). In a study carried out on farms in Nakuru District, Rift Valley Province, Kenya, the total cost of control and economic losses due to ticks and tick-borne diseases in the same year (1987) was estimated at US$ 13.64 per animal per annum (Young, Mutugi, Kariuki, Lampard, Maritim, Ngumi, Linyonyi, Leitch, Ndungu, Lesan, Mining, Grootenhuis, Orinda & Wesonga, 1992), a substantial amount as kenya had a cattle population of 11.9 million in that year (Kariuki, 1990).

A return to the intensive tick control programme of the pre independence war era (Perry, Mukhebi, Norval & Barrett, 1990) is currently almost as expensive to Zimbabwe as it is to the Kenya. For example, during the 1988/89 financial year, the government of Zimbabwe spent an estimated US$ 9 million (Perry *et al.*, 1990). Thus, in their report to the Director of Veterinary Services, Zimbabwe, Perry *et al.* (1990) proposed that alternative tick-borne disease control strategies which make less intensive use of acaricides and rely more on controlled immunization and the development of natural immunity to tick-borne diseases should be considered.

The estimated costs given above for Kenya and Zimbabwe are examples of the high costs involved in the control of tick-borne diseases. Other countries within the

theileriosis region of eastern, central and southern Africa also suffer similar losses. This is mainly due to the reliance on the conventional tick-borne disease control methods which are basically acaricidal application and chemotherapy. The benefits of new control methods that are safer, cheaper, more effective and sustainable have been discussed elsewhere (Young *et al.,* 1988; Mukhebi, 1992; Norval, Barrett, Perry & Mukhebi, 1992; Musoke, 1993).

1.7 Objectives

The aim of this study was two-fold: i) to fill in some gaps in the poorly understood areas of the life cycle of *Theileria parva* in the tick vector, and ii) to use the data generated to develop parameter estimates for use in quantitative models of theileriosis dynamics in the tick vectors.

The specific objectives were as follows:

1. To compare the prevalence and abundance of *T. parva* infections established in nymphal and adult ticks.
2. To find out whether tick stocks from different regions vary in their ability to transmit different stocks of *T. parva* under laboratory conditions.
3. To investigate the rate and success of development of *Theileria* parasites under different environmental conditions related to development of the larval/nymphal and nymphal/adult tick vectors.
4. To develop models of *T. parva* infections in rhipicephalid ticks by using data generated from experiments designed to achieve objectives 1, 2 and 3 above.

CHAPTER 2

MATERIALS AND METHODS

2 MATERIALS AND METHODS

2.1 *Theileria parva* stocks

Four stocks of *T. parva* (Muguga, Kiambu 5, Marikebuni and Boleni) were used to infect larval and nymphal ticks in these experiments. *Theileria parva* (Muguga) has been well characterized and had been maintained in the laboratory for at least 20 years by serial tick-cattle passages (Bailey, 1960; Brocklesby, Barnett & Scott 1961). More recently it has been maintained as tick-derived stabilates (Radley, Brown, Burridge, Cunningham, Pierce & Purnell, 1974; Dolan, Young, Losos, McMillan, Minder & Soulsby, 1984). It is thought to have been isolated in the Kiambu District of Kenya (Young & Leitch, 1981; Büscher & Otim, 1986). *Theileria parva* Kiambu 5 was isolated from a field case in the Kiambu District in the Central Province of Kenya in 1971 and maintained in stabilate form since (Irvin, Purnell, Brown, Cunningham, Ledger & Payne, 1974). *Theileria parva* (Marikebuni) was originally isolated from Marikebuni village (standard grid reference EG2459 (Survey of Kenya, 1972)), Kilifi District in the Coast Province of Kenya in 1981 by collecting adult *Rhipicephalus appendiculatus* ticks from the field and applying them to susceptible cattle in the laboratory. Nymphal *R. appendiculatus* were fed on the sick animals and the resultant ground up supernate of the adult ticks cryopreserved in liquid nitrogen (Irvin, *et al.*, 1983; Minami, Spooner, Irvin, Ocama, Dobbelaere & Fujinaga, 1983). Characterization of the parasite using monoclonal antibody profiles was carried out by Minami, *et al.*, (1983). *Theileria parva* Boleni was originally isolated by feeding nymphal ticks on an adult cow named "Horse" originating from Boleni Farm, Goromonzi, near Harare, Zimbabwe in July, 1978. The farm whose standard grid reference is UR3323 (Surveyor-General, Zimbabwe, 1985), had experienced a severe

outbreak of theileriosis in January, 1978. The cow had theilerial parasites in the blood and high levels of antibody to *T. parva* schizont antigen by the IFA test (Lawrence & MacKenzie, 1980). The parasite has been cryopreserved in stabilate form since isolation and has been a subject of several studies (Lawrence & Mackenzie, 1980; Uilenberg, Perié, Lawrence, de Vos, Paling & Spanger, 1982; Irvin, Morzaria, Munatswa & Norval, 1989; Koch, 1990).

2.2 Tick stocks

Seven stocks of *Rhipicephalus appendiculatus* and one stock of *Rhipicephalus zambeziensis* from eastern, central and southern Africa were used for these experiments.

Rhipicephalus appendiculatus Muguga (RAM) - originally established as a colony from collections from Muguga, Kiambu, District Kenya and maintained under laboratory conditions since 1952 using the methods described by Bailey (1960).

Rhipicephalus appendiculatus Kiambu (KEN KIA) - obtained in early 1992 as engorged female ticks collected from cattle in Kiambu District, Kenya.

Rhipicephalus appendiculatus Mashonaland West (ZIM WM) - obtained early in 1992 from Mashonaland West, Zimbabwe as engorged female ticks collected from cattle.

Rhipicephalus appendiculatus Lake McIlwaine (McILW) - obtained from the Lake McIlwaine Recreational Park near Harare, Zimbabwe in 1976 and has been maintained under laboratory conditions since (Short & Norval, 1981).

Rhipicephalus appendiculatus Eastern Province (ZAM EP) - obtained as engorged females collected from cattle from Chipata Eastern Province, Zambia in early 1992.

Rhipicephalus appendiculatus Southern Province (ZAM SP) - obtained as engorged females collected from cattle from Monze and Hufwa, Southern Province, Zambia in early 1992.

Rhipicephalus zambeziensis Nuanetsi (R. ZAM) - obtained from collections from the Nuanetsi Ranch near Mwenezi in south-eastern Zimbabwe in 1987 and maintained under laboratory conditions since.

KEN KIA, ZAM EP, ZAM SP and ZIM WM were used for experiment either in their first or second laboratory generation

2.3 Animals

Cattle used were either *Bos indicus* type (Boran breed) or *Bos taurus* type (Friesian breeds), which were negative for antibodies against *T. parva* schizonts using the indirect fluorescent antibody test (Goddeeris, Katende, Irvin & Chumo, 1982). These were obtained from the ILRAD Experimental Animal Unit and each weighed approximately 150 kg.

The rabbits used were pure bred New Zealand whites obtained from the Small Animal Unit at ILRAD, Veterinary Research Laboratories, Kabete or National Agricultural Farm, Ngong. Each weighed approximately 2 kg.

2.4 Infection of ticks

2.4.1 Acute Theileria parva *Muguga infections*

Boran steers were infected by subcutaneous injection below and in front of the right ear with 1 ml of stabilate 3087 of the Muguga stock of *T. parva* diluted to 1/20 according to the methods of Musoke, Morzaria, Nkonge, Jones & Nene (1992). Rectal temperatures of the Boran cattle were taken daily in the morning to monitor development of the parasite. Needle biopsy smears were prepared from the right parotid lymph node daily from day 5 after infection, smeared on glass slides, fixed in methanol, stained in Giemsa's and examined for the presence of schizonts under oil emersion (x400 magnification). Similarly, the contralateral prescapular lymph node was sampled and thin blood smears from peripheral ear veins prepared daily from day 10 after infection and examined for the presence of schizonts and piroplasms respectively. Piroplasm parasitaemia was estimated by counting the number of infected erythrocytes per 1,000. The animals were treated daily with a short-acting oxytetracycline formulation (Agricyl-100, Pacific Animal Health, Anthony Products Co., Arcadia, California) at a dose of 10mg/kg body weight on days 10 to 14 after stabilate inoculation to prolong infection (Bailey, 1960; Young, Dolan, Morzaria, Mwakima, Norval, Sherriff & Gettinby, in preparation a). Uninfected larval or nymphal rhipicephalid tick stocks were applied in cloth bags glued to the ears or shaven areas on the backs of the cattle respectively on day 13 post infection so that they fed to repletion during the parasitaemic phase of infection. It has been shown that the site of tick feeding does not influence the level of infection developing in subsequent instars (Büscher & Tangus, 1986). The replete ticks were collected daily and kept separately at 24°C and approximately 85% relative humidity (RH) for 28 days to complete their moult. They were then transferred to 20°C, 85% RH for 14

days to harden before processing for assessment of infection. The southern and central African ticks were kept for a further 3 weeks at 85% RH, at 14h light: 10h dark, and a diurnally fluctuating temperature of 16-26°C to break the behavioural diapause (Young, Mwakima, Ochanda, Dolan, Norval, Perry, Berkvens & Pegram, in preparation b) before being assessed for infection.

2.4.2 Acute Theileria parva *Boleni infections*

For *Theileria parva* Boleni infections, dairy breed cattle negative for *T. parva* schizont antigen using the indirect fluorescent antibody test (Goddeeris *et al.* 1982), were infected by subcutaneous injection in front of the right ear with either 1 ml or 4 ml of *T. parva* Boleni stabilate GU79/1 (Pegram, James, Bamhare, Dolan, Hove, Kanhai & Latif, in press) and treated intramuscularly with dexamethasone (Biomeda, Dublin, Eire) at 0.2mg/kg body weight on day 8, 10, 12 and 14 after infection. Development of the parasite was thereafter monitored as described for acute *T. parva* Muguga infections in Section 2.4.1. Application of uninfected nymphae onto the cattle and subsequent processing of the ticks was also done as described in Section 2.4.1.

2.5 Assessment of ticks for *Theileria parva* infections using Feulgen's stain

In order to examine tick batches for infections, nymphal and adult ticks were first fed on rabbits ears for 3 or 4 days respectively so that the majority of *T. parva* sporoblasts matured into sporozoites. The ticks were enclosed in linen bags (approximately 10x15 cm) attached onto rabbit ears using zinc oxide adhesive plaster (Leukoplast, BDF Beiersdorf AG Hamburg, Germany). The ticks were then removed from ears and the salivary glands dissected out carefully using fine-pointed surgical blade (No. 11 BS

2982, Paragon, Sheffield, England) and forceps. Ten salivary glands were spread individually on each glass slide and allowed to dry. These were fixed in 90% methanol for 5 minutes, dried and then hydrolysed in 5N HCl for 1 hour. The excess acid was drained off and the glands stained in Feulgen's stain for 1 hour (Büscher & Otim, 1986). They were subsequently washed thoroughly under running tap water, dried then examined under 100 times magnification to assess the number of acini infected with the theilerial parasites per individual tick. Thirty males and 30 females were examined for each adult batch while 60 were examined for each nymphal batch. In the case of infected nymphae, an assessment had to be carried out to establish the duration of feeding that was most suitable in detecting the highest infections by removing the ticks from the rabbit ears and assessing infection from days 0-5 after application. The technique for efficient and accurate dissection of nymphae was developed for the first time and with practise it was shown that it could be achieved efficiently (Chapter 3).

2.6 Statistical analysis

Differences in prevalence and abundance of infection between the tick instars and gender were analysed using the logistic and Poisson regression analysis respectively This analysis is described in detail in Chapter 6.

CHAPTER 3

COMPARISON OF THE TRANSMISSION OF *THEILERIA PARVA* BETWEEN LARVAL/NYMPHAL AND NYMPHAL/ADULT INSTARS OF *RHIPICEPHALUS APPENDICULATUS*.

3.1 SUMMARY

The efficiency of transmission of *Theileria parva* by nymphal and adult *Rhipicephalus appendiculatus*, as judged by infections developing in their salivary glands was compared. When larval and nymphal ticks were fed concurrently on cattle undergoing acute *T. parva* Muguga infection, the resultant nymphal ticks developed a slightly lower prevalence of infection than the resultant adult ticks. However, the abundance of infection was 5-20 times higher in the adult ticks. When cattle infected with the Boleni stock were used, adult ticks produced relatively high infections but infection was not detectable in the nymphae. When cattle that were carriers of two stocks of *T. parva* were used as the source of infection, the infection rates developing in adult ticks were much higher than those developing in nymphal ticks. Thus, nymphae may develop higher infections if fed as larvae on cattle experiencing acute infections compared to those feeding on cattle with sub-acute infections or carrier cattle. Sporozoites could be seen in the salivary glands of nymphal ticks from day 2/3 after attachment to the host. In adult females, sporozoites were usually produced from day 4 after attachment of the ticks and the infected "e" cell of the type III acini were greatly enlarged. Similar development occurred in nymphae but sporozoites were produced a day earlier. The infection pattern was different in males where infection levels were lower than in females and the development of sporozoites was more irregular. It is suggested that differences in infection levels between females, males and nymphs could be explained largely by variations in the structures of the salivary glands in the different instars and sexes.

The relationships between infection prevalence and abundance between male, female and nymphal ticks are explored further in Chapter 6.

3.2 INTRODUCTION

For logistic reasons, *Theileria parva* nymphal/adult transmission has been studied in some detail, whereas larval/nymphal transmission has been a subject of few studies. However both Lounsbury (1904) and Theiler (1905a), who first demonstrated the transmission of *T. parva* by ticks, were able to show larval/nymphal transmission. It is well known that more nymphae feed on cattle than adults, and Short and Norval (1981) have suggested the ratio of 10 nymphae to 1 adult. The probable reason why larval/nymphal transmission has been neglected in comparison to nymphal/adult transmission has been a general belief that adult ticks which have engorged as nymphae on infected cattle are largely responsible for transmission of infection in the field (Purnell, Boarer & Peirce, 1971). In addition the relatively small size of the nymphae compared to adults makes them difficult to count on cattle, difficult to handle and to assess in terms of their *T. parva* infections.

While few studies have been carried out on larvae/nymphae transmission, even fewer show comparisons with nymphae/adult transmission so that the relative role of larvae/nymphae transmission remains unclear. Those studies which have been published reveal confusing results. Reichenow (1940) suggested that higher prevalence and abundance of infection occurred in nymphae than adults which fed concurrently as larvae and nymphae on the same infected animal. This was not confirmed by Purnell *et al.* (1971) who found lower prevalence and abundance of infection in nymphae than adults. Instead they suggested that a batch of nymphae would contain more parasites per gramme than a corresponding batch of adults. However further work by Purnell *et al.* (1973) did not confirm this as they found that prevalence and abundance of infection in nymphae was much lower. Barnett

and Bailey (1955) also reported transmission by nymphae and found that when between 5 and 10 nymphae were applied, lethal infections occurred in half the cattle compared to 100% using adult ticks (Brocklesby *et al.* 1961). In Zimbabwe, Lawrence, Norval and Uilenberg (1983) demonstrated nymphal transmission of *T. parva* with *Rhipicephalus zambeziensis,* but failed to transmit the same infections with nymphal *R. appendiculatus* even when 1000 nymphae were used. In contrast, Koch (1990) and Koch, Kambeva, Norval, Ocama and Munatswa (1993) successfully transmitted *T. parva* in Zimbabwe using larvae/nymphae *R. appendiculatus* transmission when large numbers of nymphae was used for transmission.

Following an initial infection, it is believed that a proportion of cattle become carriers, that is, they remain infectious to ticks without acquiring further infection (Young *et al.,* 1986). Medley *et al.* (1993a) have demonstrated that *T. parva* carrier cattle are important in the maintenance, hence epidemiology of the parasite as they may constitute the majority of the cattle population in many areas. A further aspect of this study is to determine the relative role of larvae in acquiring infection from carrier animals.

In this study the relative roles of nymphae, adult females and adult males of *R. appendiculatus* in the transmission of *T. parva* was compared. These studies were carried out under experimental conditions but it was expected they would give some insight into processes occurring under field conditions. In addition the differences in salivary gland structure between instars was investigated for its role in the relative levels of infection developing in different instars. Events within the salivary glands during sporogony were also studied.

3.3 MATERIALS AND METHODS

3.3.1 Assessment of nymphal ticks for infection

It is a standard practice to feed adult ticks for 4 days on rabbits before they are dissected and their salivary glands assessed for *Theileria parva* infections (Purnell & Joyner, 1968; Purnell, Young, Brown, Burridge & Payne, 1974). The best day to assess nymphal ticks for salivary gland infections in comparative studies was determined. Four nymphal tick batches which had fed to repletion on 4 cattle 19 days after infection (of the cattle) were applied separately on rabbit ears. Sixty nymphae from each group were assessed before and after 1, 2, 3 or 4 days of attachment using the standard method of Feulgen's staining (Section 2.5). All unattached nymphae were discarded a day after they were applied on the rabbit ears to feed.

3.3.2 Assessment of tick sample size

Rhipicephalus appendiculatus Muguga tick batches of different infection levels, which had fed to repletion as nymphae on routinely *T. parva* Muguga-infected cattle (Section 2.4.1) were assessed for infection in their salivary glands after Feulgen's staining (Section 2.5). Two batches, having an abundance of infection of 40 or 4 infected acini in the female ticks respectively (categorised arbitrarily as "highly" or "lowly" infected), were selected. Five replicates of each of the two categories of ticks were assessed for infection either after Feulgen's staining or as live specimens under interference contrast microscopy (Young, Leitch, Stagg & Dolan, 1983a).

The prevalence and abundance of infections within each category of ticks and between the two assessment procedures were compared.

3.3.3 Interference contrast microscopy

Salivary gland preparations were either examined with or without fixing in 2.5% gluteraldehyde. The glands were individually placed in a drop of phosphate buffered physiological saline (PBS), and a cover slip gently pressed on each. Their structure was then studied using Nomarski Interference Contrast Microscope (Leitz, West Germany) (Young *et al.*, 1983a).

3.3.4 Comparison of the infectivity of acute *Theileria parva* Muguga and *T. parva* Boleni infections for *Rhipicephalus appendiculatus* nymphs and adults

Four Boran steers, numbered BJ104, BJ105, BJ111, and BJ112, were inoculated with *T. parva* Muguga stabilate as described in Section 2.4.1. Uninfected larvae and nymphae of *R. appendiculatus* Muguga stock were applied to the cattle and allowed to feed to repletion. The replete ticks were kept to moult after which they were processed for Feulgen's staining (Section 2.5) and their degree of infection determined and recorded.

In a second experiment, dairy cattle BK258 and BK314 were infected with 4 ml and 1ml respectively of *T. parva* Boleni stabilate GU79/1 and treated with dexamethasone (Section 2.4.2). Development of the parasite in the cattle was then monitored. Uninfected larvae and nymphae of *R. appendiculatus* Muguga and McIlwaine stocks were applied to the cattle on day 13 after infection and allowed to

feed to repletion during the parasitaemic phase of infection (Section 2.4). The replete ticks were then treated as described in Section 2.4 then assessed for infection (Section 2.5).

3.3.5 Comparative transmission from *Theileria parva* carrier cattle by nymphae and adult *Rhipicephalus appendiculatus*

Three Boran breed cattle, numbered BJ264, BJ267 and BJ204, were used. BJ264 and BJ267 had been inoculated subcutaneously 17 months previously with 1ml of 1/5 dilution of *T. parva* Marikebuni cloned stabilate 3292 and treated with a short-acting formulation of oxytetracycline on day 10 to 14 after infection. BJ204 had been inoculated 6 months previously with 1 ml of *T. parva* Kiambu 5 stabilate. All three animals had positive antibody titres in the IFA test and harboured piroplasms in their erythrocytes. *Theileria parva* Muguga could not be used in this study since carrier state has not been demonstrated in this stock (Barnett, 1968; Dolan, 1986; Bishop, Sohanpal, Kariuki, Young, Nene, Baylis, Allsopp, Spooner, Dolan & Morzaria, 1992). Approximately 2000 larvae and 1600 nymphae of *R. appendiculatus* Muguga were applied to each animal as described in Section 2.4 and were allowed to feed to repletion. The replete ticks were maintained by the standard method (Section 2.4.1) and assessed for infection.

3.3.6 The number and distribution of different acini types in nymphae and adult *Rhipicephalus appendiculatus*

The salivary glands of twenty uninfected nymphae, males and female *R. appendiculatus* of the Muguga stock were dissected out and fixed *in situ* individually

in 2% gluteraldehyde in 0.1M cacodylate phosphate buffer, pH 7.2 for 30 min. The salivary glands were washed thoroughly in PBS before mounting individually on a glass slide in a drop of PBS. A coverslip was placed on the salivary gland and pressed gently to spread out the acini. These were examined using the interference contrast optics and the number of easily recognisable acini from type I to IV were counted per nymph, male and female. The distribution of the salivary gland acini types was studied by interference contrast examination and by transverse sections of plastic embedded salivary glands stained in toluidine blue 2% examined under the standard light microscope.

3.3.7 Comparative development of sporozoites in nymphae and adult ticks

Larvae and nymphae of the Muguga stock of *R. appendiculatus* were fed to repletion on *T. parva* Muguga parasitaemic Boran cattle. They were allowed to moult and harden by the standard method. The ticks were applied to rabbit and removed after feeding for 1, 2, 3, and 4 days. The salivary glands were dissected out and divided into two, one half of which was prepared for Feulgen's staining and the other half for ultrastructural studies. The half salivary gland was processed according to the methods of Shaw, Tilney and Musoke (1991) for electron microscope examination. Only the salivary glands showing high infections on examination of the Feulgen's stained half were examined under the electron microscope.

3.4 RESULTS

3.4.1 Days of feeding for assessment of *Theileria parva* infections in nymphal ticks

The total number of infected salivary gland acini detected using Feulgen's stain increased as the number of days of nymphal feeding increased (Figure 3.1). The increase continued until three days after feeding commenced when the highest prevalence and abundance of infections were detected. By the fourth day of nymphal feeding, the number of infected salivary gland acini detected had started diminishing. Thus, infected nymphae were routinely fed on rabbits for 3 days before assessment.

3.4.2 Assessment of tick sample size

Results of the average prevalence and abundance of infection in the adult *R. appendiculatus* Muguga feeding to repletion on cattle infected with *T. parva* Muguga assessed after either Feulgen's staining or interference contrast microscopy procedure are given in Table 3.1. As expected, female ticks in a batch generally had higher infection levels than male ticks. However, except for one sample in the highly infected group, differences in infection levels between batches and the two assessment procedures in each respective group were not significant ($P > 0.05$).

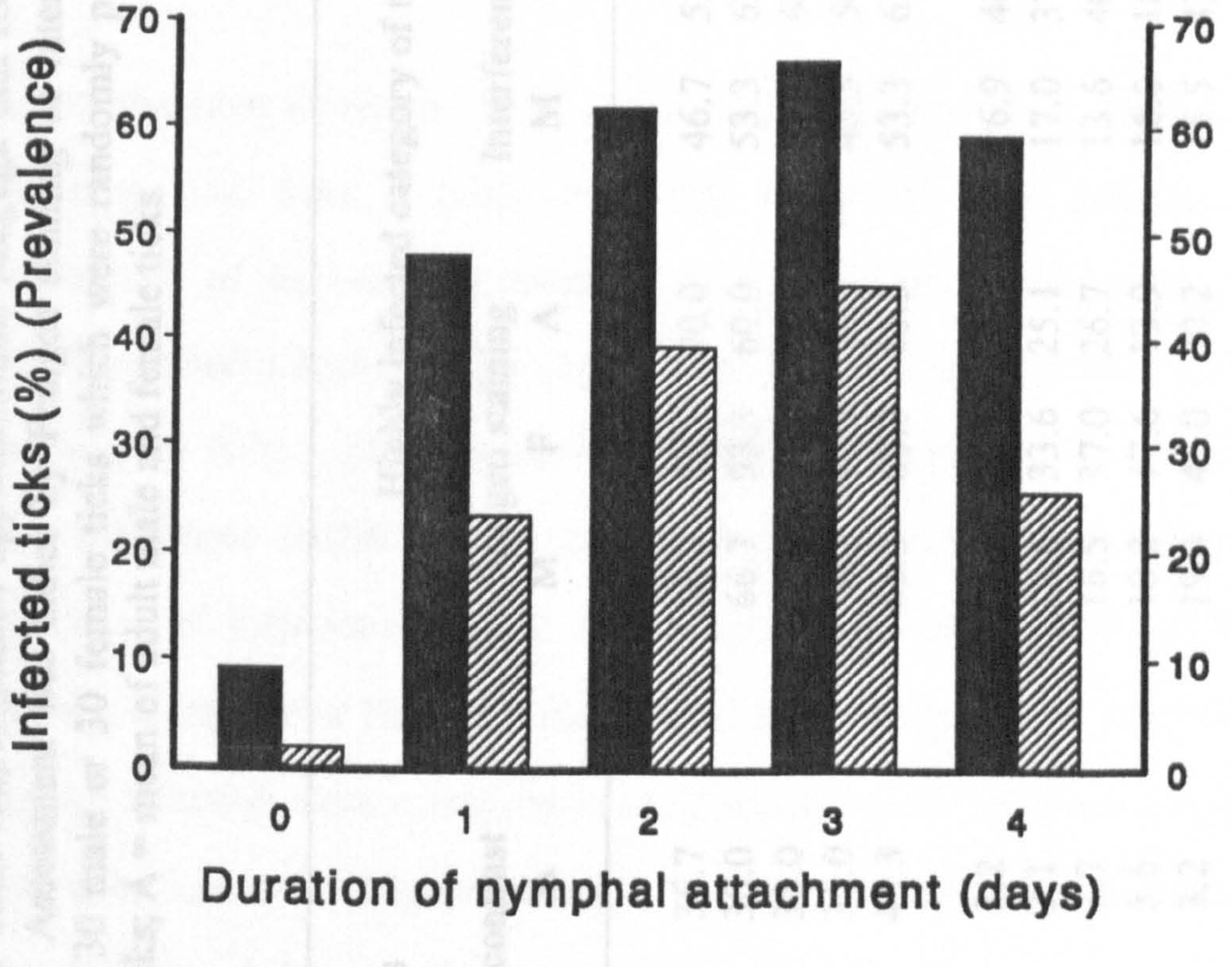


Figure 3.1. Prevalence (■) and mean abundance (▨) of infections developing in four batches of *Rhipicephalus appendiculatus* Muguga nymphae fed to repletion as larvae on four cattle suffering acute *Theileria parva* Muguga infection. Each batch contained 60 nymphal ticks.

Table 3.1. Mean prevalence and abundance of infections in adult *Rhipicephalus appendiculatus* Muguga that fed to repletion as nymphae on cattle infected with *Theileria parva* Muguga. Assessment was either by Feulgen staining or interference contrast microscopy. Each of the five replicates sampled contained 30 male or 30 female ticks which were randomly picked from each respective category. M = adult male ticks; F = adult female ticks; A = mean of adult male and female ticks.

	Lowly infected category of ticks						Highly infected category of ticks					
	Feulgen staining			Interference contrast			Feulgen staining			Interference contrast		
	M	F	A	M	F	A	M	F	A	M	F	A
Prevalence of infection	43.3	56.7	50.0	33.3	40.0	36.7	56.7	83.3	70.0	46.7	53.3	50.0
	43.3	70.0	56.7	20.0	40.0	30.0	66.7	53.3	60.0	53.3	63.3	58.3
	36.7	56.7	46.7	26.7	43.3	35.0	53.3	56.7	55.0	36.0	60.0	48.3
	33.3	56.7	45.0	33.3	36.7	35.0	56.7	63.3	60.0	43.3	50.0	46.7
	36.7	46.7	41.7	46.7	40.0	43.3	63.3	63.3	63.3	53.3	63.3	58.3
Abundance of infection	2.3	4.1	3.2	2.3	4.2	3.2	15.2	40.3	27.7	16.9	40.4	28.7
	2.1	4.5	3.3	1.9	4.2	3.1	16.6	33.6	25.1	17.0	37.6	27.3
	1.6	3.9	2.8	2.3	5.0	3.7	16.5	37.0	26.7	13.6	40.7	27.2
	2.6	4.9	3.8	2.5	4.7	3.6	10.2	17.6	13.9	16.0	18.7	17.4
	1.9	4.1	3.0	2.0	4.3	3.2	19.4	41.0	30.2	15.5	43.9	29.7

3.4.3 Comparison of the infectivity for *Rhipicephalus appendiculatus* nymphae and adults of *Theileria parva* Muguga acquired from acutely infected cattle

The comparative infectivity of *T. parva* Muguga is shown in Table 3.2 and Figure 3.2 using data from 4 cattle from day 16 to 20 after infection. The mean parasitaemia of the cattle increased with time and the mean prevalence in the tick batches reached a maximum on day 18 after infection in adult ticks and on day 19 in the nymphal ticks. The prevalence of infection established in nymphal ticks were lower than those established in corresponding adult tick batches. The ratio between prevalence of infections in adult and nymphal ticks varied between 0.9 and 2.4 in different comparative batches. However if the abundance and intensity of *T. parva* Muguga infection were compared in adult and nymphal tick batches it was found that adult ticks had much higher values. The same pattern of abundance was seen in adult and nymphal ticks with the maximum abundance occurring on day 18 in adult ticks and on day 19 in nymphae ticks. The ratio between abundance of infection in adults and nymphae was greater with a minimum of 3.4 and maximum of 24.1. The prevalence and abundance of *T. parva* infections established in the male ticks were lower than those established in corresponding female ticks.

3.4.4 Comparison of the infectivity for *Rhipicephalus appendiculatus* nymphae and adults of *Theileria parva* Boleni acquired from acutely infected cattle

Two stocks of ticks, *R. appendiculatus* Muguga and McIlwaine were used. Relatively high infections, as judged by the prevalence and mean abundance of infection, were seen in adult ticks but no infection could be detected in any comparable batch of nymphae (Table 3.3 and Figure 3.3).

Table 3.2. Levels of infection developing in *Rhipicephalus appendiculatus* nymphae and adults which fed to repletion as larvae and nymphae respectively on four steers suffering acute *Theileria parva* Muguga infections. AA/NN = the ratio of prevalence and abundance of infection of adult ticks to that of nymphal ticks; * = death of bovine host; P = bovine piroplasm parasitaemia.

Days after infection of bovine host	Tick instar, gender or ratio	Steer BK 97			Steer BJ 283			Steer BJ 318			Steer BJ 435		
		P	Pre-valence	Abun-dance	P	Pre-valence	Abun-dance	P	Pre-valence	Abun-dance	P	Pre-valence	Abun-dance
16	Nymphs	8.2	26.7	0.7	5.6	18.3	0.3	6.6	28.3	0.8	2.2	28.3	1.1
	Males		30.0	2.9		16.7	2.2		23.3	2.4		40.0	15.9
	Females		53.3	24.5		36.7	9.3		50.0	28.7		60.0	53.3
	AA/NN		1.6	18.7		1.5	17.3		1.3	20.8		1.8	18.9
17	Nymphs	19.4	36.7	2.6	12.6	28.3	1.3	9.8	45.0	3.5	8.4	31.7	3.0
	Males		56.7	25.0		36.7	9.3		53.3	24.9		70.0	56.0
	Females		80.0	73.8		83.3	39.5		73.3	40.6		83.3	90.6
	AA/NN		1.9	19.0		2.1	19.5		1.4	9.5		2.4	24.1
18	Nymphs	26.0	56.7	7.9	14.6	53.3	5.1	11.2	55.0	6.6	9.2	50.0	5.0
	Males		73.3	61.1		86.7	39.4		73.3	67.3		93.3	36.9
	Females		90.0	121.8		93.3	72.6		80.0	38.5		76.7	60.6
	AA/NN		1.3	11.5		1.7	10.9		1.4	8.1		1.7	9.8

Table 3.2 (contd.).

Days after infection of bovine host	Tick instar, gender or ratio	Steer BK 97 P	Steer BK 97 Pre-valence	Steer BK 97 Abun-dance	Steer BJ 283 P	Steer BJ 283 Pre-valence	Steer BJ 283 Abun-dance	Steer BJ 318 P	Steer BJ 318 Pre-valence	Steer BJ 318 Abun-dance	Steer BJ 435 P	Steer BJ 435 Pre-valence	Steer BJ 435 Abun-dance
19	Nymphs	28.1	73.3	13.4	16.0	70.0	9.9	14.2	68.3	10.7	10.2	53.3	11.2
	Males		56.7	40.0		73.3	42.8		66.7	16.6		86.7	53.2
	Females		76.7	58.4		86.7	72.8		93.3	106.0		93.3	100.2
	AA/NN		0.9	3.7		1.1	5.9		1.2	5.7		1.7	6.8
20	Nymphs	34.0	51.7	7.3	*	41.7	3.7	16.0	65.0	10.0	10.0	55.0	7.7
	Males		56.7	22.3		80.0	30.9		76.7	21.8		76.7	46.0
	Females		86.7	118.9		80.0	59.1		83.3	46.5		86.7	95.2
	AA/NN		1.4	9.6		1.9	12.2		1.2	3.4		1.5	9.2

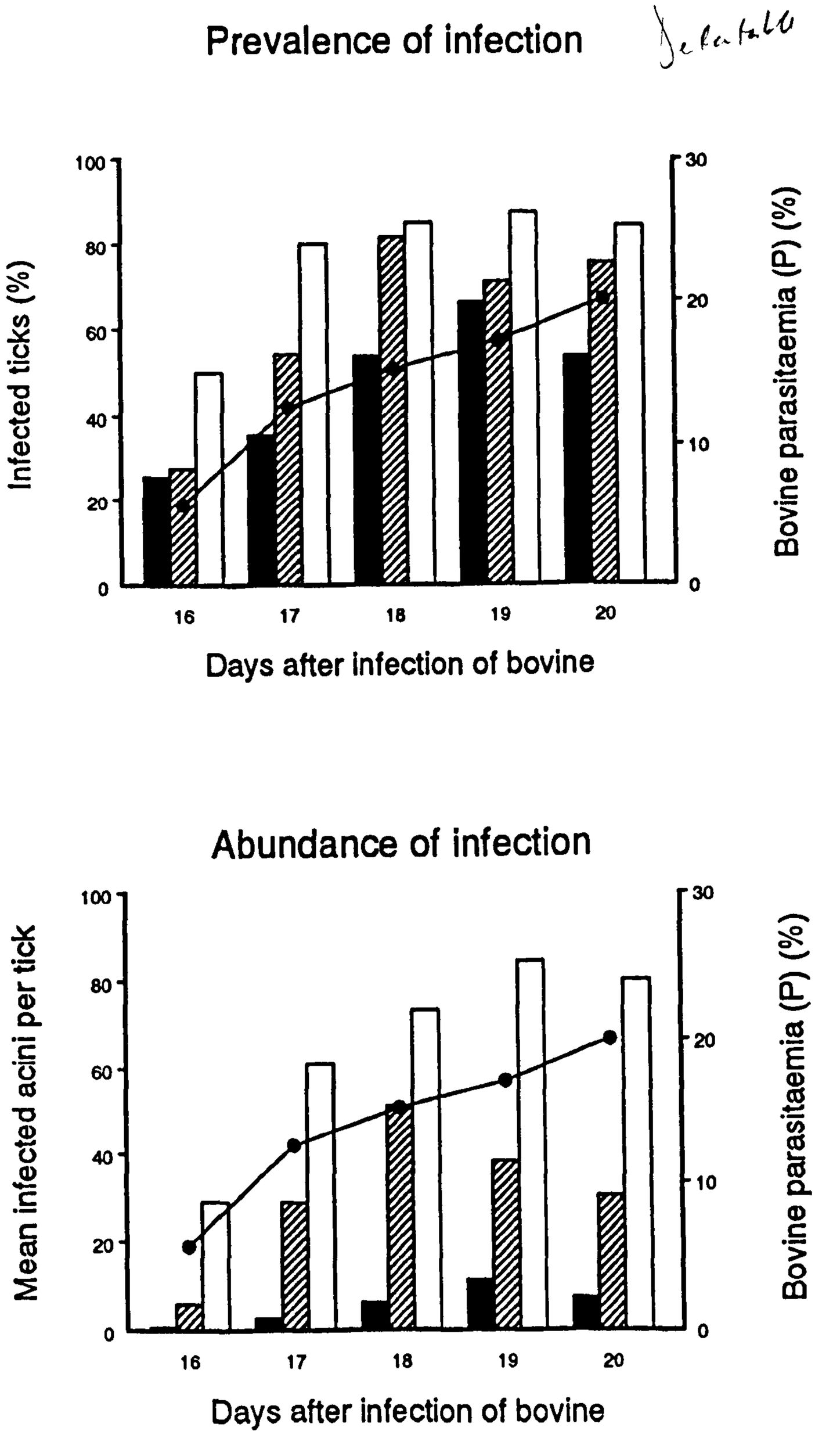


Figure 3.2. Levels of infections developing in *Rhipicephalus appendiculatus* Muguga nymphae (■) and adult males (▨) and females (▭) feeding to repletion as larvae and nymphae respectively on four cattle suffering acute *Theileria parva* Muguga infections. Five tick batches dropping replete from each bovine host on days 16 to 20 after sporozoite stabilate inoculation were assessed for infection. Mean bovine piroplasm parasitaemia (●) over the five days is given.

Table 3.3. Levels of infections developing in *Rhipicephalus appendiculatus* nymphae and adults feeding to repletion as larvae and nymphae respectively on cattle suffering acute *Theileria parva* Boleni infections. N.B. The parasitaemia (%) for the 4 days were respectively: BK 258 0.3, 0.6, 0.2, <0.1; BK 314 0.8, 3.6, 3.8, 2.8.

Tick stock	Tick instar or gender	Measure of infection	Infection levels of ticks after infection of bovine host							
			Day 17		Day 18		Day 19		Day 20	
			BK258	BK314	BK258	BK314	BK258	BK314	BK258	BK314
RAM	Nymphs	Prevalence	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Abundance	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Males	Prevalence	50.0	66.7	13.3	13.3	0.0	3.3	0.0	10.0
		Abundance	12.1	19.3	0.5	0.4	0.0	0.1	0.0	0.2
	Females	Prevalence	73.3	70.0	26.6	40.0	10.0	23.3	3.0	6.6
		Abundance	24.6	57.9	3.7	23.5	4.0	3.3	0.3	0.4
	Adults	Prevalence	61.7	68.3	20.0	26.7	5.0	13.3	1.2	8.3
		Abundance	18.4	38.6	2.1	12.0	2.0	1.7	0.2	0.3
McILW	Nymphs	Prevalence	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		Abundance	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Males	Prevalence	56.7	56.7	50.0	83.3	36.7	76.7	30.0	63.3
		Abundance	5.3	17.4	14.9	38.3	3.3	8.3	2.1	18.2
	Females	Prevalence	76.7	66.7	50.0	83.3	56.7	76.7	23.3	80.0
		Abundance	22.9	23.8	8.8	122.5	6.1	25.8	1.9	66.8
	Adults	Prevalence	66.7	61.7	50.0	83.3	46.7	76.7	26.7	71.7
		Abundance	14.1	20.6	11.9	80.4	4.7	17.1	2.0	42.5

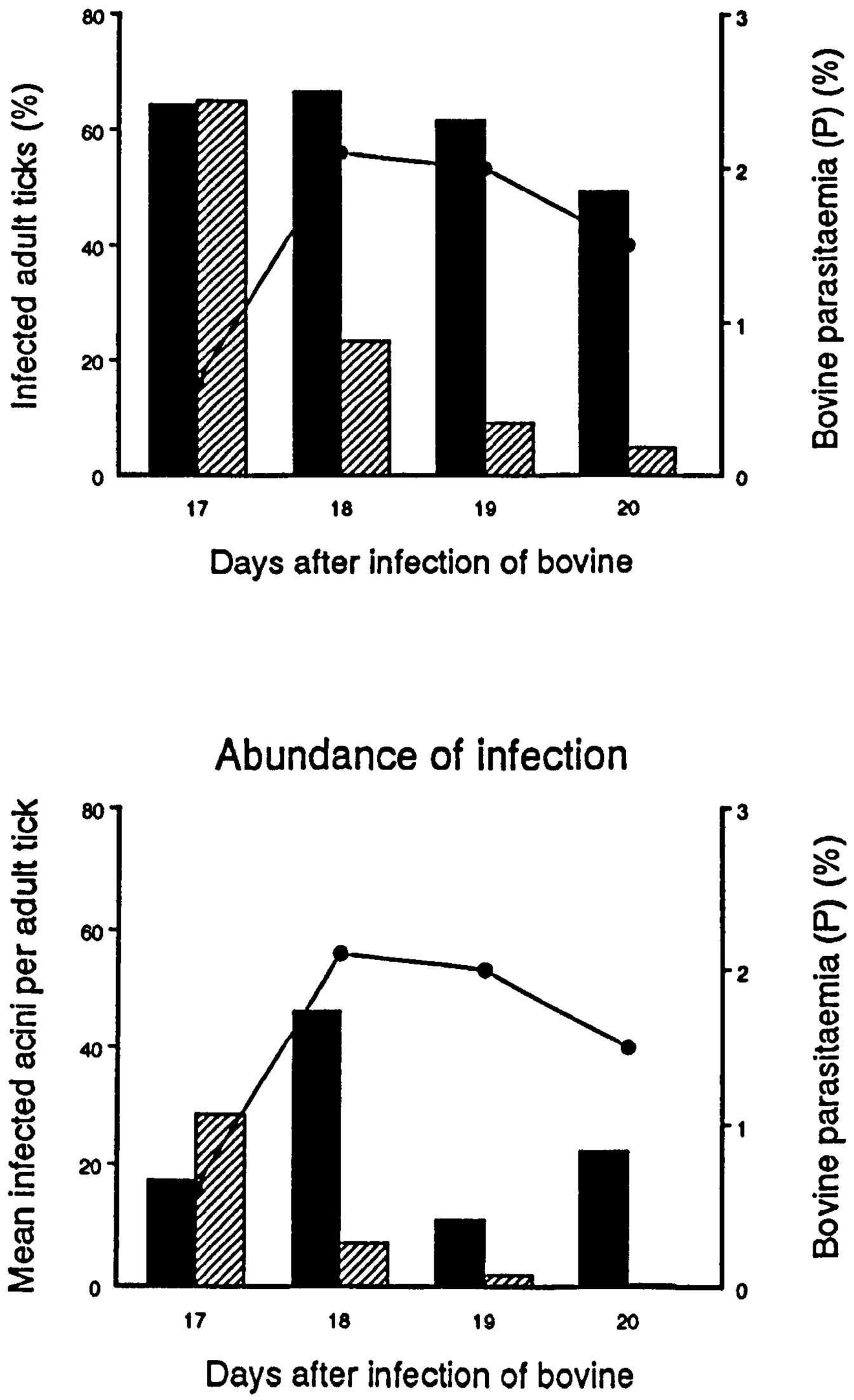


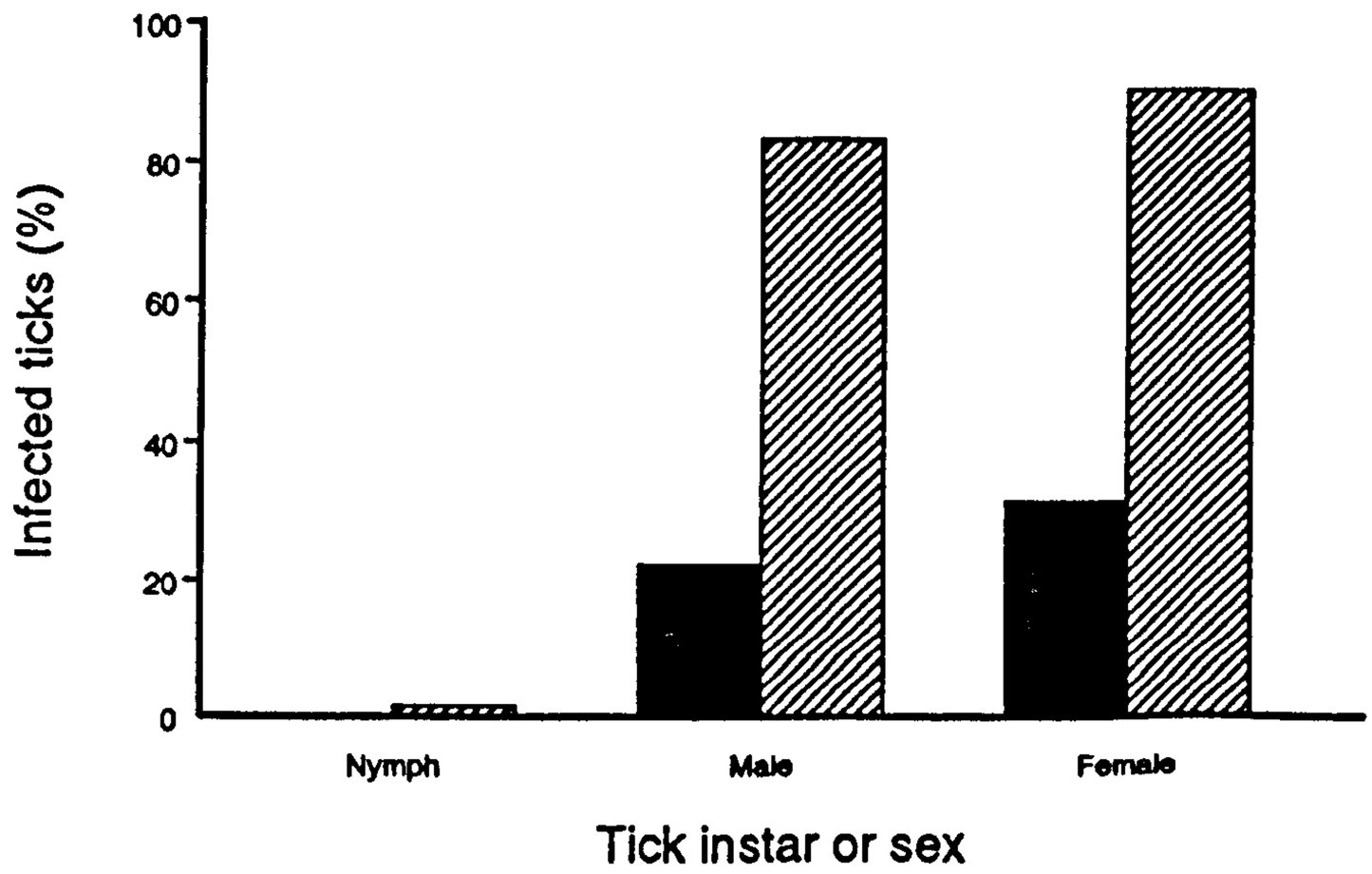
Figure 3.3 Levels of infection developing in the Muguga (■) and McIlwaine (▨) stocks of *Rhipicephalus appendiculatus* adults feeding to repletion as nymphae on two cattle suffering acute *Theileria parva* Boleni infections. Four tick batches dropping replete from each bovine host on days 17 - 20 after sporozoite stabilate inoculation were assessed for infection. The mean bovine piroplasm parasitaemia (●) over the four days is given.

3.4.5 Comparative transmission from *Theileria parva* carrier by nymphal and adult *Rhipicephalus appendiculatus* ticks.

Of the 3 cattle used in this experiment only 2 were demonstrated to be carriers at the time of experiment (Table 3.4). All adult tick batches that fed to repletion on animal BJ204, infected with *T. parva* Kiambu 5, were infected. In contrast, no nymphal tick batch showed infection. When animal BJ267 was used as a source of infection which had recovered from *T. parva* Marikebuni, it was found that adult ticks produced higher prevalence (3.2 times) and abundance (46.5 times) of infection compared to those that fed to repletion on animal BJ204. However, the nymphal ticks failed on 3 out of 5 batches to show any detectable infections. The two nymphal tick batches, which did develop infections, had much lower infections than the corresponding adult ticks (Figure 3.4).

Table 3.4. Levels of infection developing in *Rhipicephalus appendiculatus* Muguga nymphae and adults feeding to repletion as larvae and nymphae respectively on carrier cattle that had been infected at least 6 months previously with various stocks of *Theileria parva.* N = nymphal ticks; M = adult male ticks; F = adult female ticks; A = mean of adult male and female ticks.

Tick batches	Measure of infection	Bovine host BJ 204 (Kiambu 5)				Bovine host BJ 264 (Marikebuni)				Bovine host BJ 267 (Marikebuni)			
		N	M	F	A	N	M	F	A	N	M	F	A
Day 1	Prevalence(%)	0.0	26.7	33.3	30.0	0.0	0.0	0.0	0.0	5.0	86.7	100.0	93.3
	Abundance	0.0	0.7	0.8	0.7	0.0	0.0	0.0	0.0	0.1	31.8	65.3	48.5
Day 2	Prevalence(%)	0.0	23.3	20.0	21.6	0.0	0.0	0.0	0.0	0.0	86.7	86.7	86.7
	Abundance	0.0	0.4	0.5	0.5	0.0	0.0	0.0	0.0	0.0	9.7	34.4	22.1
Day 3	Prevalence(%)	0.0	16.7	36.7	26.7	0.0	0.0	0.0	0.0	0.0	77.0	80.0	78.3
	Abundance	0.0	0.3	1.1	0.7	0.0	0.0	0.0	0.0	0.0	13.1	37.8	25.5
Day 4	Prevalence(%)	0.0	23.3	23.3	23.3	0.0	0.0	0.0	0.0	3.3	83.3	96.7	90.0
	Abundance	0.0	0.5	0.5	0.5	0.0	0.0	0.0	0.0	0.1	15.5	57.1	36.3
Day 5	Prevalence(%)	0.0	13.3	16.7	15.0	0.0	0.0	0.0	0.0	0.0	83.3	90.0	86.7
	Abundance	0.0	0.2	0.2	0.2	0.0	0.0	0.0	0.0	0.0	10.0	37.0	23.5
Day 6	Prevalence(%)	0.0	26.7	43.3	35.0	0.0	0.0	0.0	0.0	-	-	-	-
	Abundance	0.0	0.7	1.8	1.2	0.0	0.0	0.0	0.0	-	-	-	-
Day 7	Prevalence(%)	0.0.	26.7	46.7	36.7	0.0	0.0	0.0	0.0	-	-	-	-
	Abundance	0.0	0.3	1.4	0.9	0.0	0.0	0.0	0.0	-	-	-	-



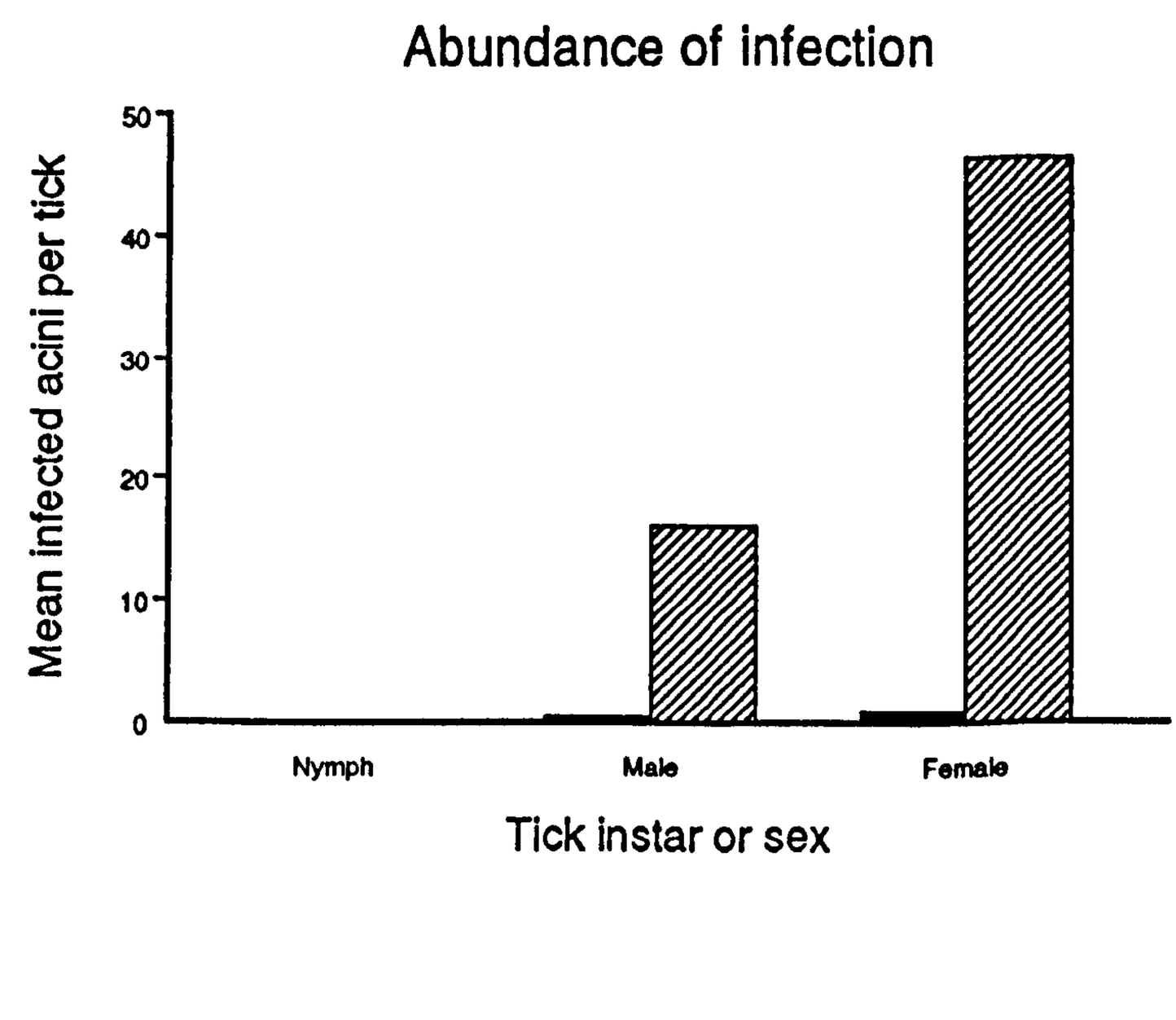


Figure 3.4. Levels of infection developing in *Rhipicephalus appendiculatus* Muguga nymphae and adults feeding to repletion as larvae and nymphae respectively on two carrier cattle infected at least six months earlier with *Theileria parva* Kiambu 5 (■) or *Theileria parva* Marikebuni (▨). Seven tick batches of each instar dropping replete over 7 days from the former bovine host and 5 from the latter were assessed for infection.

3.4.6 Numbers of different acini in salivary glands of instars and sexes

Table 3.5 shows the number of acini in salivary glands of instars and sexes of *R. appendiculatus* Muguga. The glands are made up of 3 types of acini plus an additional type in males. The salivary glands of nymphal ticks are much smaller than adults, although the general structure is similar to the adult female. The mean number of acini in nymph is only 127 compared to 2164 in male ticks and 2233 in female ticks. There were considerable variations in the number of type III acini in the instars between individual ticks (shown in brackets in Table 3.5).

3.4.7 Structure of salivary glands in relationship to infection

The structure of salivary gland acini in the three tick instars is shown in Figures 3.5 and 3.6. Type III acini in all the instars appear to be of approximately the same size. Transverse sections of the salivary glands of nymphal and adult male and female ticks are shown in Figure 3.6. It can be seen that the main salivary gland collecting duct is central and type I acini, as well as some type II acini, arise directly from the duct. The type III acini are distally arranged in all cases. In the male the type IV acini are distally arranged replacing some of the type III acini.

Table 3.5. Mean number of different types of acini in *Rhipicephalus appendiculatus* salivary glands (n = 20). The range of the type III acini between individual tick instars or gender is given in brackets.

Tick instar or gender	Type of acini				
	I	II	III	IV	Total
Nymphs	19	21	87 (68-101)	-	127
Males	246	443	1346 (1111-1896)	129	2164
Females	176	321	1736 (1300-2109)	-	2233

Figure 3.5. Fresh preparations of segments of salivary glands of *Rhipicephalus appendiculatus* Muguga showing the relative sizes of type III acini in nymphal ticks fed for 3 days (A), adult female ticks fed for 4 days (B) and adult male ticks fed for 4 days (C).

The micrographs were taken under Nomarski interference contrast microscopy at the same magnification (x 140). Note that the type III acini in all cases were approximately the same size.

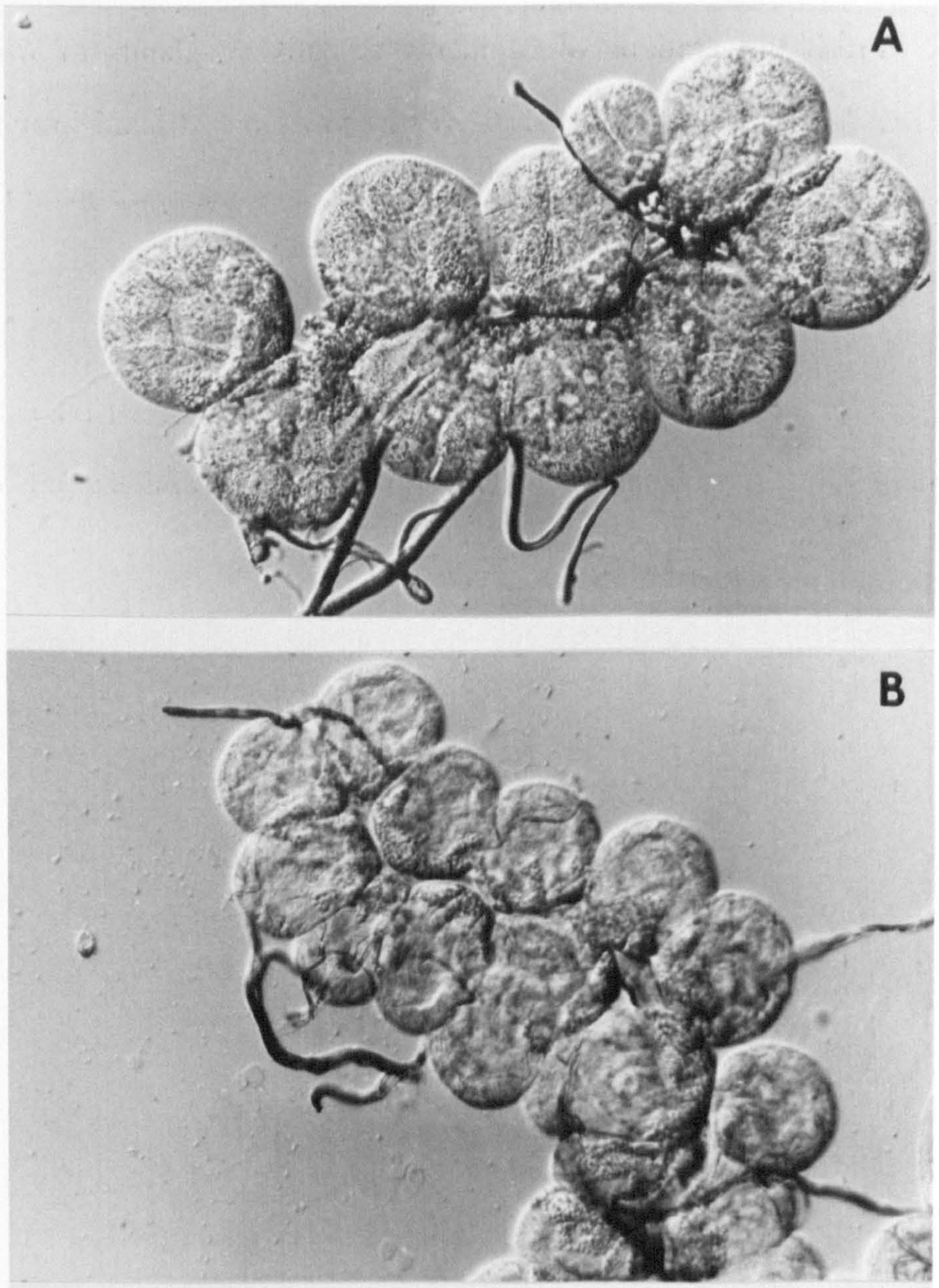
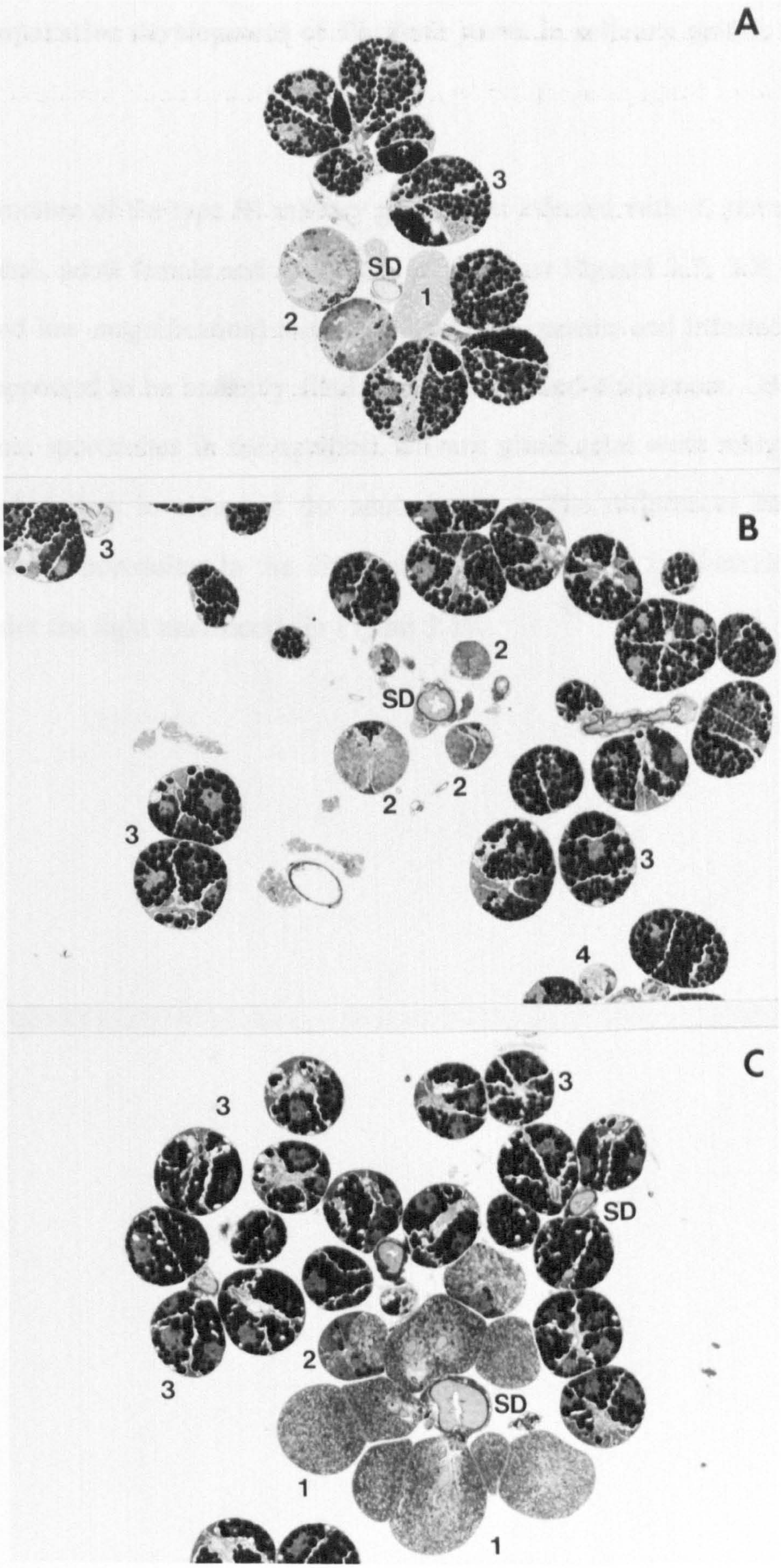

A
B
C

Figure 3.6. Transverse sections of the salivary glands of 3-day fed *Rhipicephalus appendiculatus* Muguga nymphae (A), adult male (B) and adult female (C).

The preparations were sectioned at 1μm after electron microscopy fixation and Toluidine blue staining. 1 = type I acini; 2 = type II acini; 3 = type III acini; 4 = type IV acini; SD = main or secondary salivary gland duct. Note the distal position of the type III and type IV acini in the male salivary gland and the proximal position of the type I and type II acini to the main salivary gland ducts. All micrographs at the same magnification (x 100).

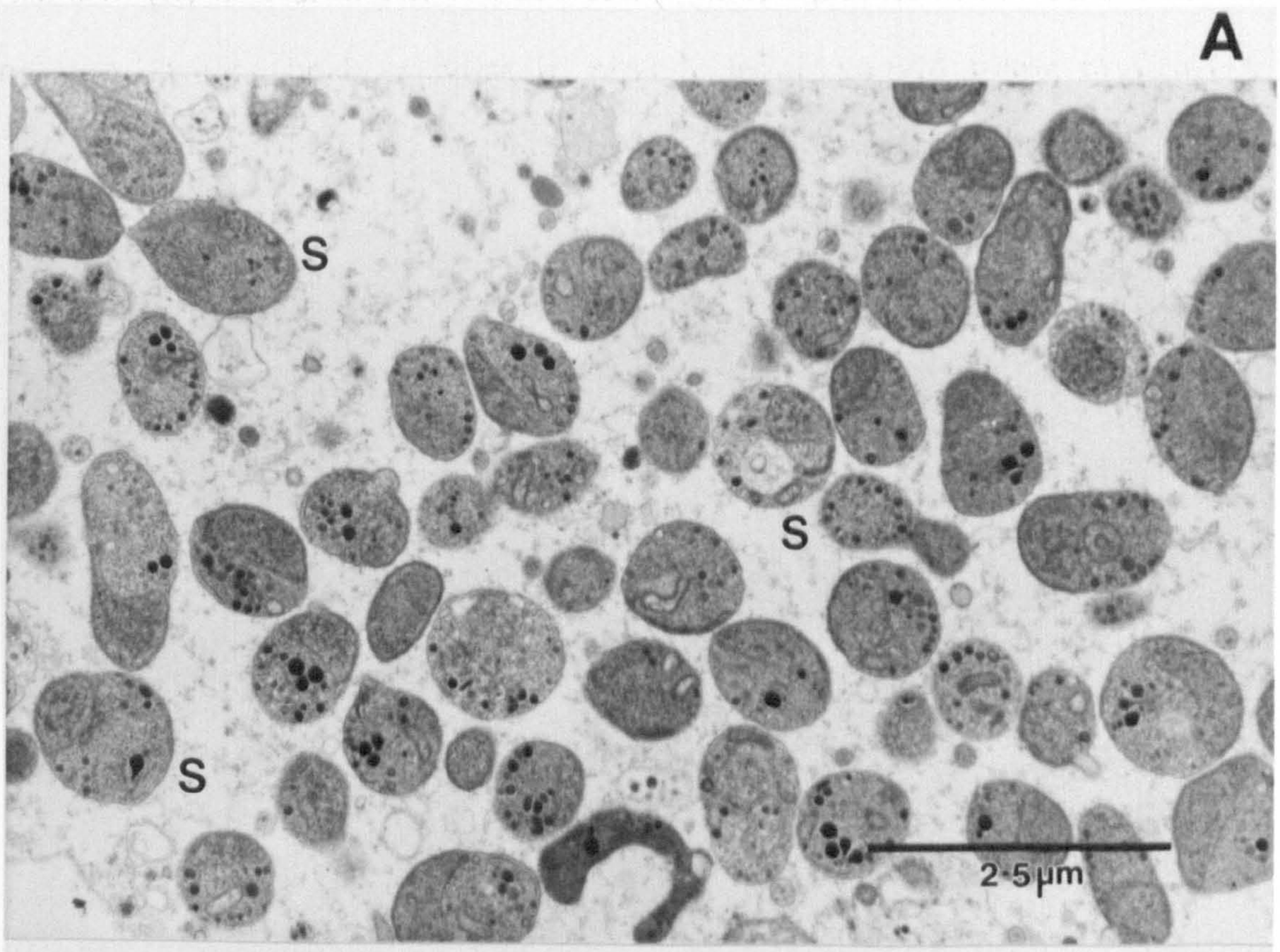

A
3
SD
1
2
B
3
2
SD
2
2
3
3
4
C
3
3
SD
2
3
SD
1
1

3.4.8 Comparative development of *Theileria parva* in salivary glands of instars and sexes

The ultrastructure of the type III salivary gland acini infected with *T. parva* Muguga from nymphal, adult female and male ticks is shown in Figures 3.7, 3.8, 3.9 (each at a high and low magnification) and 3.10. Both the parasite and infected acini cell structures appeared to be basically similar with no marked differences. However, it did seem that sporozoites in the nymphal salivary gland acini were marginally less densely packed than in either of the adult instars. The differences between the development of sporozoites in the different instars and sexes is illustrated by thin sections under the light microscope in Figure 3.10.

Figure 3.7. Electron micrograph at high (A) and low magnification (B) of a section of a *Theileria parva* Muguga-infected type III salivary gland acinus from female *Rhipicephalus appendiculatus* Muguga after feeding on rabbits for 4 days.

S = sporozoites; L = labyrinth.


A
S
S
S
2·5 μm

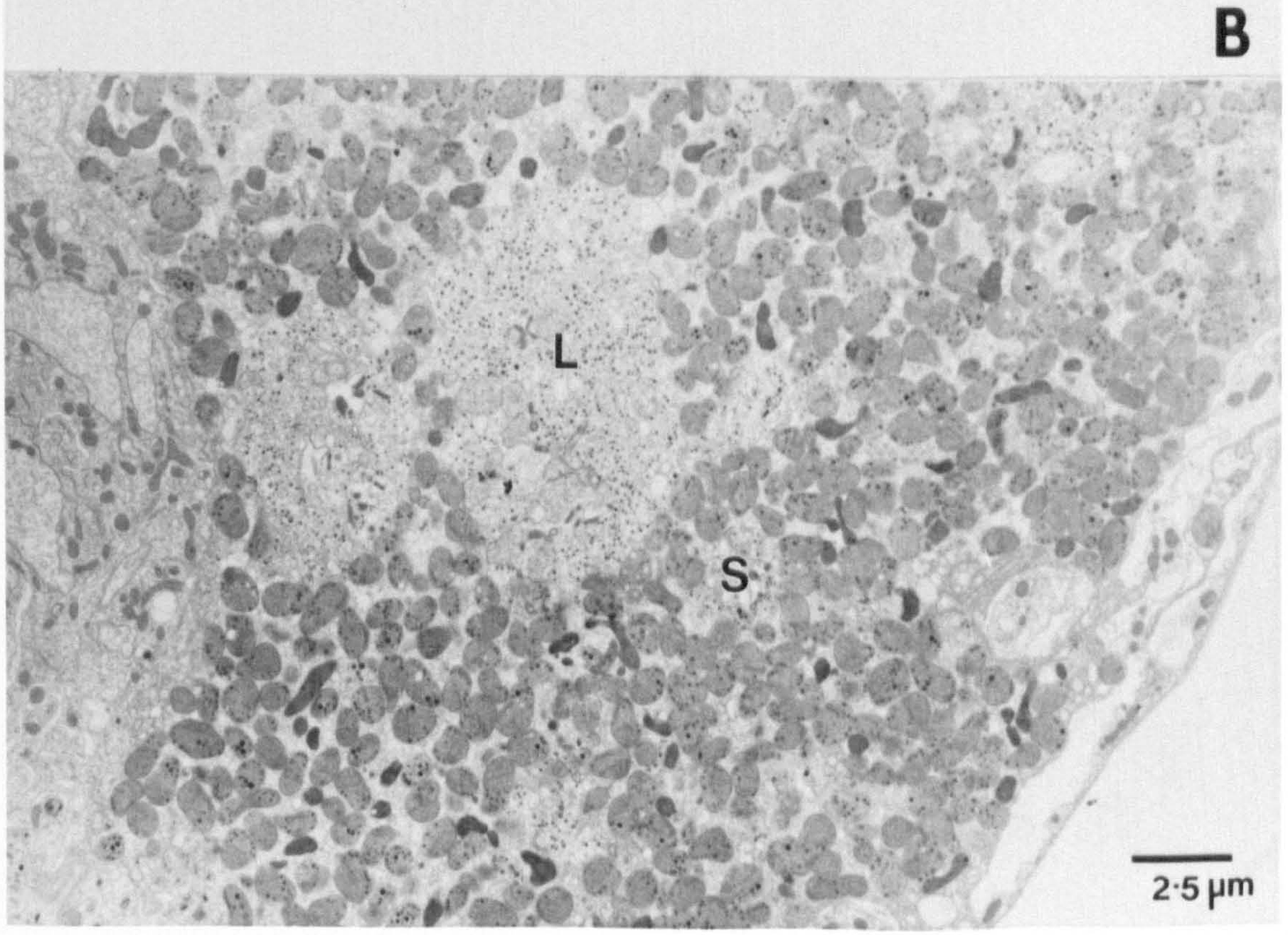
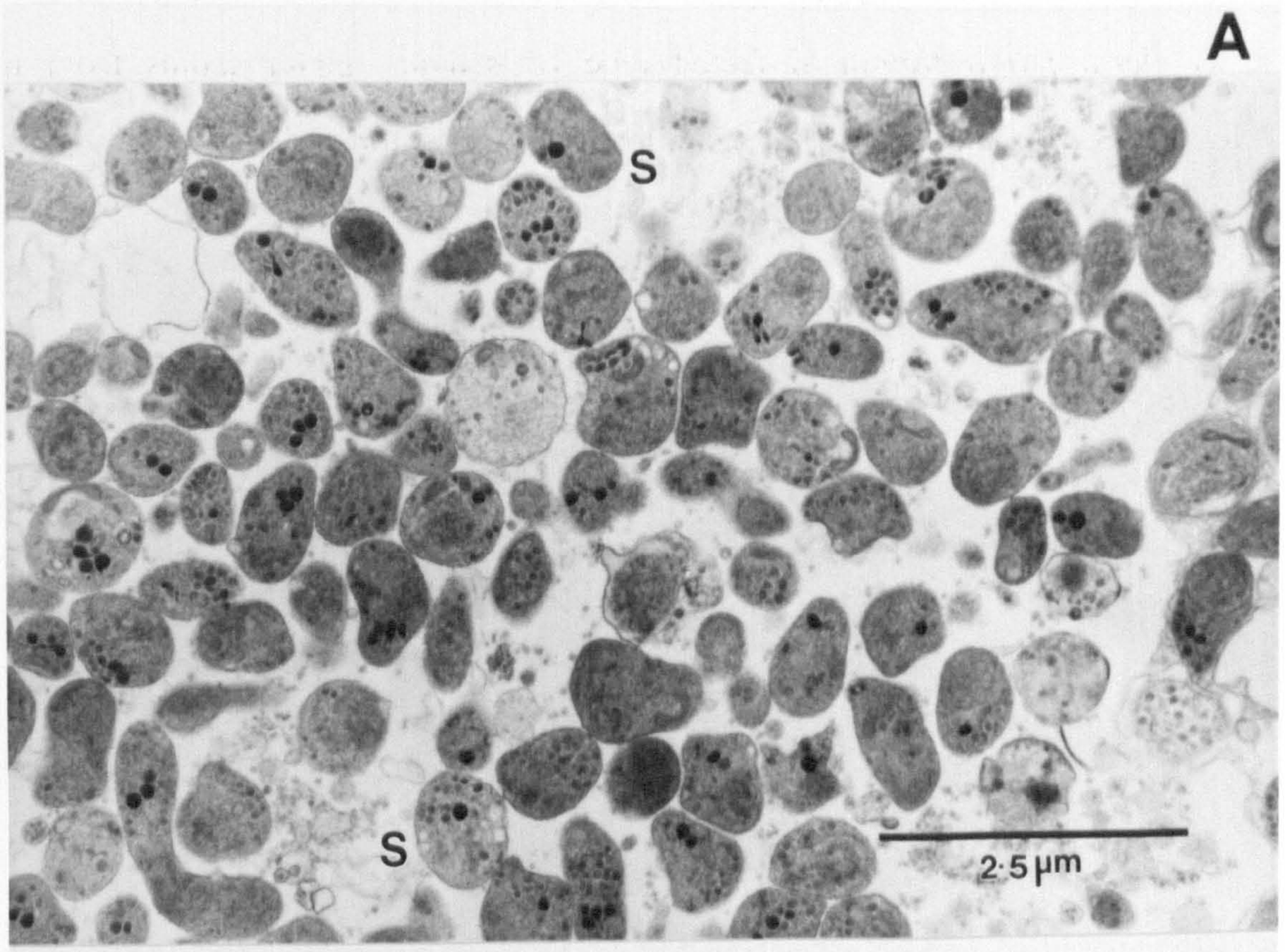

B
L
S
2·5 μm

Figure 3.8. Electron micrograph at high (A) and low magnification (B) of a section of a *Theileria parva* Muguga-infected type III salivary gland acinus from male *Rhipicephalus appendiculatus* Muguga after feeding on rabbits for 4 days.
S = sporozoites; L = labyrinth.


A
S
S
2·5 µm

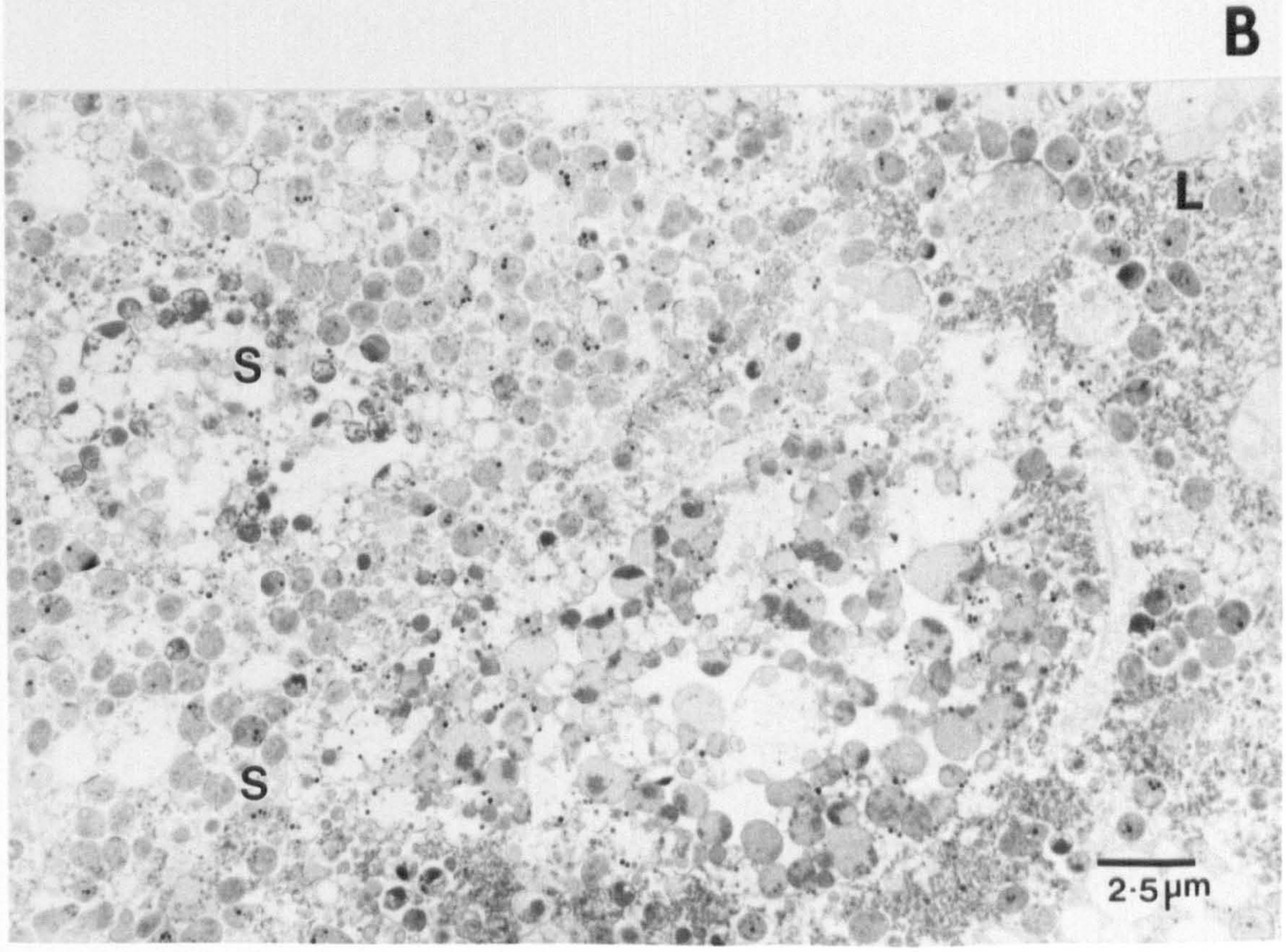
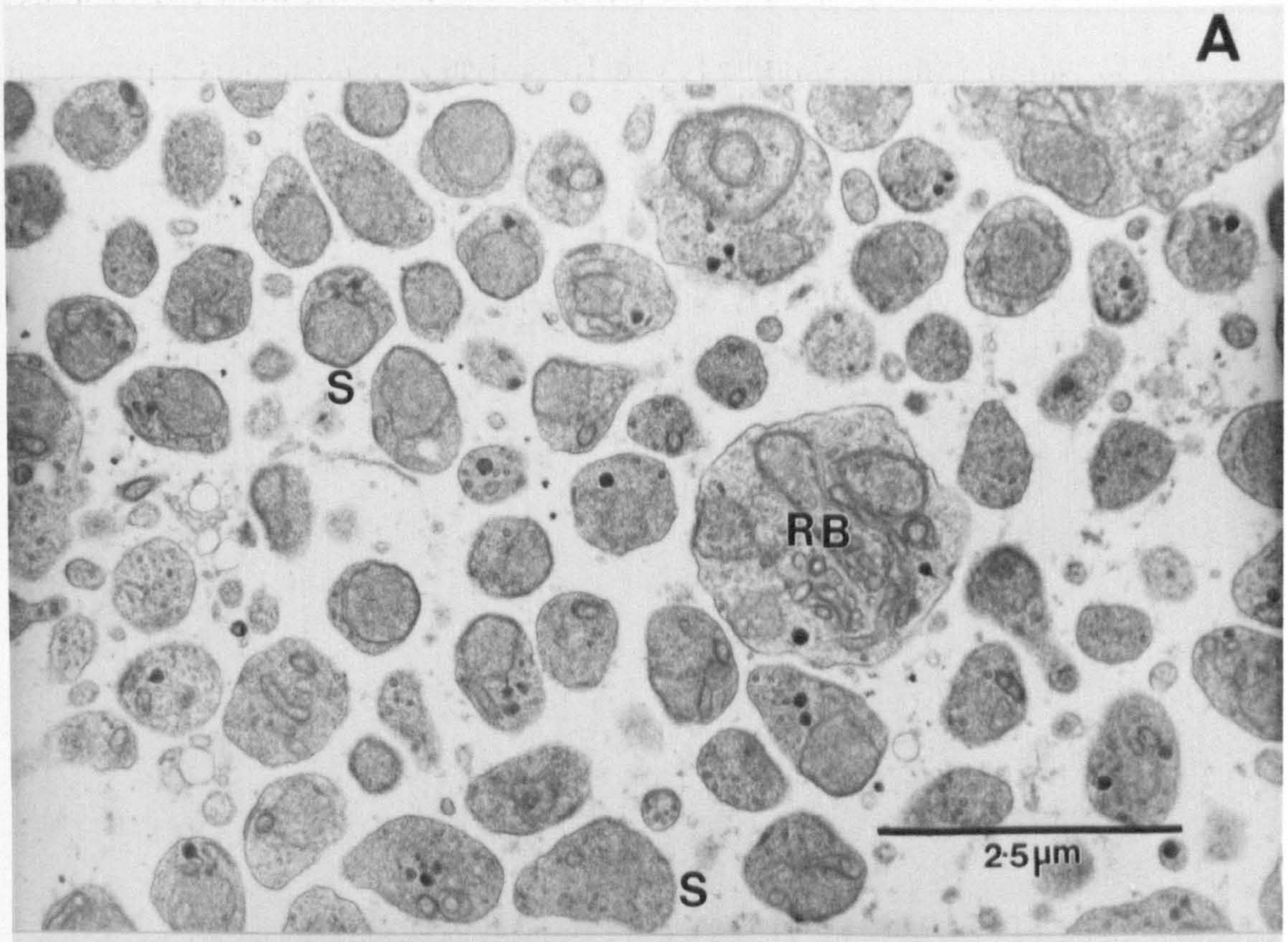

B
L
S
S
2·5 µm

Figure 3.9. Electron micrograph at high (A) and low magnification (B) of a section of a *Theileria parva* Muguga-infected type III salivary gland acinus from nymphal *Rhipicephalus appendiculatus* Muguga after feeding on rabbits for 3 days.

S = sporozoites; L = labyrinth; RB = residual body.


A
S
RB
S
2·5 µm

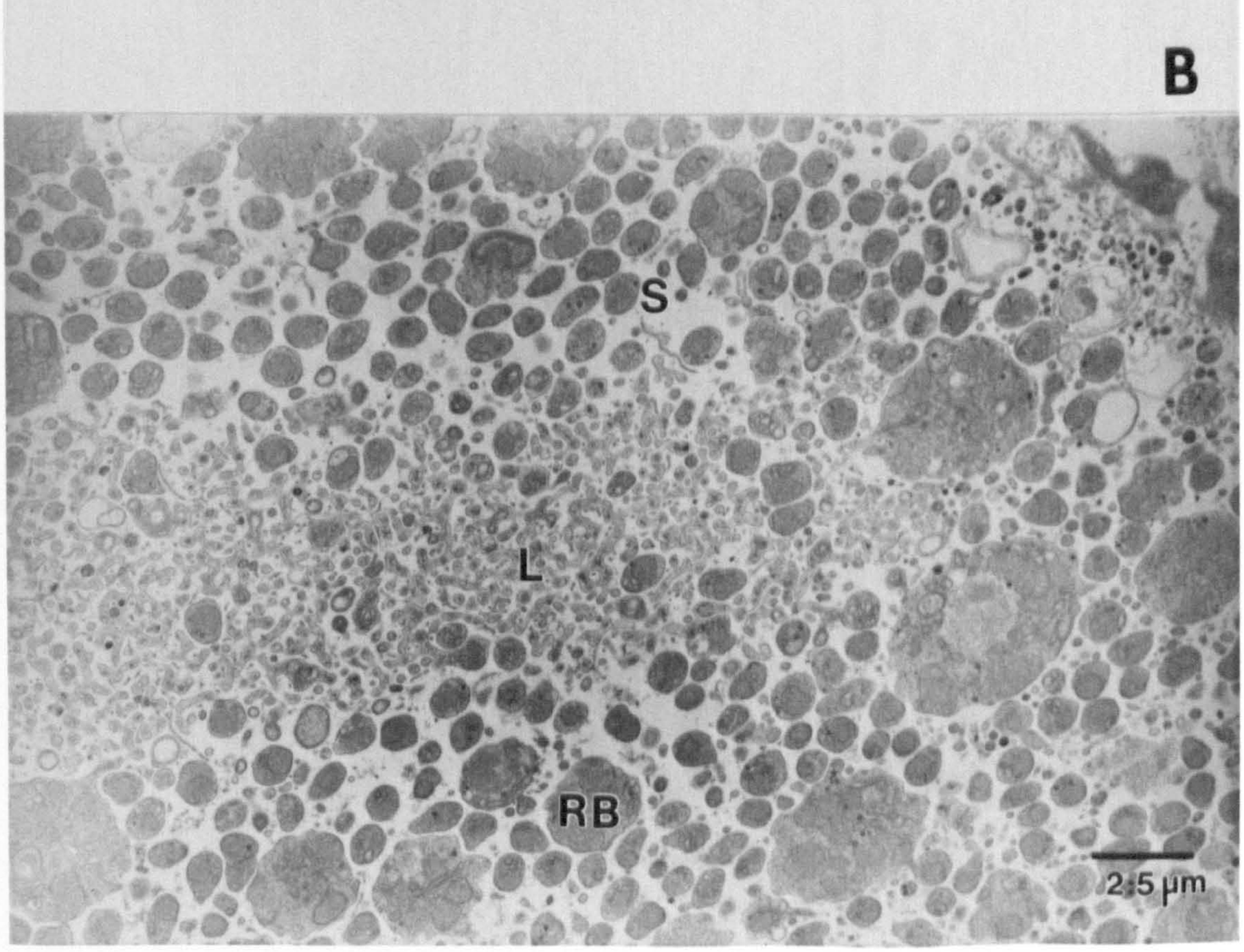
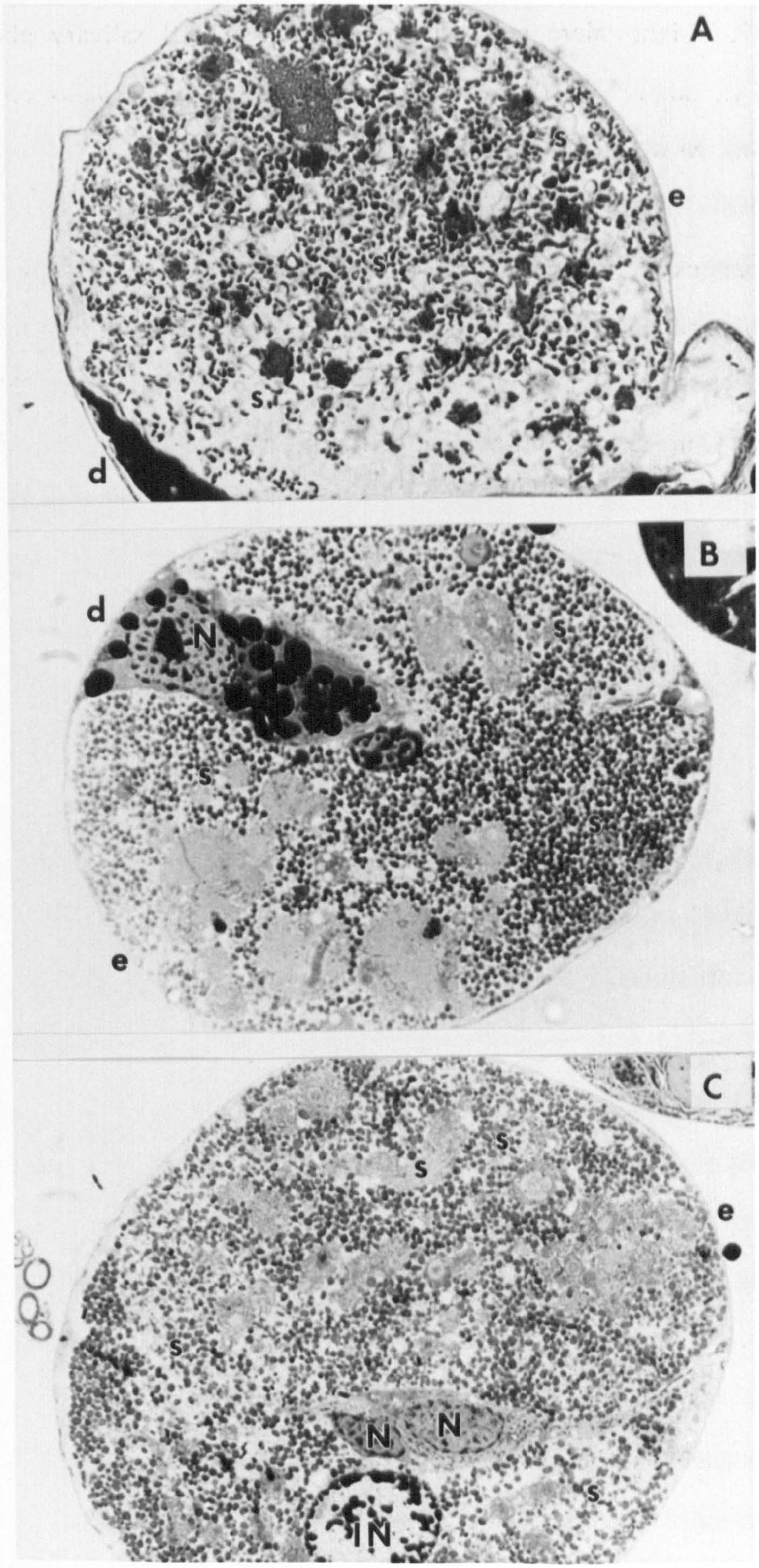

B
S
L
RB
2·5 µm

Figure 3.10. Light micrographs of sections of type III salivary gland acini of nymphal (A), adult male (B) and adult female (C) *Rhipicephalus appendiculatus* Muguga infected with sporozoites of *Theileria parva* Muguga.

The preparations were sectioned at 1 μm after electron microscopy fixation and Toluidine blue staining. S = sporozoites; e = infected "e" cell; d = uninfected "d" cell; N = nuclei of uninfected cell; IN = nucleus of infected "e" cell. All micrographs at the same magnification (x 1040).


A
e
s
s
d
B
d
N
s
s
s
e
C
s
s
e
s
N
N
N

3.5 DISCUSSION

Little published information is available on the relative roles of the instars of *Rhipicephalus appendiculatus* in the efficiency of transmission of *Theileria parva*. This information is important for studying the epidemiology and population dynamics of the parasite. This study has demonstrated that the levels of *T. parva* infections developing in larval/nymphal ticks is lower than that developing in nymphal/adult ticks. This difference in infection levels confirms the results of most previous studies (except that of Reichenow, 1940). The techniques used here to assess infections in ticks are more sensitive than those which have been used previously in some studies for assessment of infections in nymphae, where serial sections of whole nymphae were performed (Purnell *et al.* 1971; Purnell *et al.* 1974).

In acute infections of *T. parva*, a variable difference occurs in the infections developing in adults and nymphae. For *T. parva* Muguga, the differences were expressed most clearly in a large difference in abundance of infection between adult and nymphal ticks. With the *T. parva* Boleni infection from southern Africa, no infection was seen in nymphal ticks even though adult ticks showed relatively high infections. This confirms the results of Lawrence *et al.* (1983), Koch (1990) and Koch *et al.* (1993). However, in the field 10 times more nymphae than adults may feed on hosts (Short & Norval, 1981), thus nymphae may also play an important role in *T. parva* transmission.

What are the factors which make larval/nymphal transmission less efficient than larval/adult transmission? An obvious feature is that larvae imbibe much smaller

blood meals than nymphae (approximately 20 times less). In terms of prevalence of tick infection, this is not likely to be an important feature when the piroplasm parasitaemia is high but could be critical where larvae feed on cattle with low parasitaemia. This was demonstrated in carrier cattle and the *T. parva* Boleni infections in this study. The reduced number of piroplasms could limit the chances of gametes of *T. parva* fusing in the tick gut. The normally high attrition rate which is thought to occur before the kinete penetrates the salivary gland of the next instar could also reduce the number of resultant parasites in the salivary glands. The mortality of parasites is difficult to measure and most of the observations have been made on infections in the salivary glands because infection there can be accurately quantified (Büscher & Otim, 1986). Shaw & Young (in press) considered salivary gland structure in males and females in relationship to *T. parva* infection. They point out that the salivary gland acini are surrounded by thick basal lamina so it is unlikely that the kinete stage, which is not chemo-attracted to salivary glands (Bell, 1980), could detect a receptor site on the surface of the "e" cell. Receptor sites could occur on the basal membrane, as has been suggested for *Plasmodium* sporozoite infection of mosquito salivary glands (Warburg & Miller, 1991).

The number of type III acini appears to be an important feature controlling the number of acini becoming infected in the instar. Fawcett *et al.* (1982a) and Binnington, Young and Obenchain (1983) showed that the "e" cell of the type III acini became selectively infected with *T. parva*. Shaw & Young (in preparation) believed that the kinetes of *T. parva* infected acini randomly. Thus type III acini become preferentially infected because of their distal arrangement and their "e" cells because they ("e" cells) represent the largest surface area for penetration by the kinete stage.

In male ticks some of the type III acini are replaced by the type IV, which are distally placed and therefore reduce the number of type III acini available for infection. The nymphal tick has 15 times fewer "e" cells or Type III acini than the male ticks and 20 times fewer than female ticks. It was noted that the abundance of infection in nymphal ticks, even during acute *T. parva* infections, reached 15-20 times less than in the male or female ticks. When the ticks fed on cattle which produced lower parasitaemia it was noted that the infection in nymphae often became undetectable, although the prevalence in males and females could still be relatively high.

There is a nonlinear relation between prevalence and intensity of infection of *T. parva* in the tick vector similar to that found in malaria parasites in mosquitoes (Medley, Sinden, Fleck, Billingsley, Tirawanchai & Rodriguez, 1993b; Chapter 6). The distribution of parasites between vector hosts, which determines the prevalence-intensity relationship, is an important consideration if aspects of the infection in the primary host are dependent on the size of the inoculum from the vector. The determinants of the parasite distribution in the vector are likely to include, amongst other things, the parasitaemia of the infecting host and tick and parasite genetics. This should be the focus of further research.

Sporozoites in nymphal ticks are produced at least a day earlier than in adult ticks which is a necessity due to the shorter feeding period of the nymph. The feeding of the male tick is quite different from the female as it takes approximately 4 times its body weight as a blood meal compared to 200 times in female and 50 times in the nymphae. The males also detach irregularly to re-attach next to the feeding female

in order to mate. This results in the sporozoite development being much more irregular in the male tick.

Abundance of infection in nymphs, and by inference, transmission efficiency, can be up to 20 times lower than in adults. This then raises an interesting question as to whether the nymphal challenge in the field is lethal to cattle. Although the sporozoite products of one infected "e" cell from an adult tick can be lethal to a Friesian animal (Young *et al.*, 1983a), it is yet to be demonstrated that the products from one *T. parva* infected acini of the nymphal tick is lethal. One of the few studies done on comparing nymphal and adult tick challenge is by Barnett and Bailey (1955). They applied 10 nymphae from an infected batch and found that only 50% of the infections were lethal to cattle compared to 100% using 10 adult tick challenge. The implication here is that nymphal challenge would tend to cause mild infections and immunize cattle against subsequent challenge. This aspect could be partially responsible for contributing to endemic stability to *T. parva* infection seen in the field (Perry, Deem, Medley, Morzaria & Young, 1992).

It has been shown in this study that nymphae/adult transmission from carrier cattle is much more efficient than larvae/nymphae transmission. Thus, nymphal/adult transmission is likely to play a more significant role in terms of establishing acute infections in cattle, whereas larval/nymphal transmission is likely to be more important in maintenance of the parasite by generating recovered, hence carrier cattle. This occurs in Zimbabwe where seasonal occurrence of instars is seen. For example, Koch (1990) was able to detect a rise in antibody titres to *T. parva* schizont antigen in sera collected from cattle during the period when adult ticks were in diapause. This suggests that nymphae were transmitting mild infections to cattle

before the onset of the more severe adult transmitted theileriosis called "January disease" which occurs when adult ticks are infesting cattle. According to Norval *et al.* (1985), 95% of clinical cases of theileriosis occur between January and March when adults alone are infesting cattle.

These studies have given an insight to differences in larval/nymphal and nymphal/adult transmission. The concept that nymphal transmission in many cases in the field is likely to introduce a sublethal inoculum into a higher proportion of cattle than adult transmission, thus resulting in the development of immunity in a higher proportion of cattle requires further investigation. Some of these issues are addressed in Chapter 6.

CHAPTER 4

VECTOR COMPETENCE OF RHIPICEPHALID TICK STOCKS FROM DIFFERENT PARTS OF AFRICA FOR THE TRANSMISSION OF *THEILERIA PARVA* STOCKS FROM KENYA AND ZIMBABWE.

4.1 SUMMARY

The vector competence of seven *Rhipicephalus appendiculatus* and *R. zambeziensis* stocks from Kenya, Zambia and Zimbabwe in transmitting *Theileria parva* was compared. The comparison was done by feeding nymphal ticks on cattle currently infected with two stocks of *T. parva*, namely *T. parva* Muguga from Kenya and *T. parva* Boleni from Zimbabwe, and assessing the infections in the salivary glands of the resultant adult ticks. It was found that there were significant differences in the patterns of vector competence which were reproducible. *Rhipicephalus appendiculatus* Muguga from Kenya and *R. appendiculatus* Zambia Eastern Province from Zambia were the most efficient vectors of *T. parva* Muguga and *T. parva* Boleni respectively. *Rhipicephalus appendiculatus* Zambia Southern Province from Zambia and *R. appendiculatus* Zimbabwe Mashonaland West from Zimbabwe were the least efficient vectors of *T. parva* Muguga and *T. parva* Boleni respectively. The difference in abundance of infection between the most and least efficient vectors for *T. parva* Muguga and *T. parva* Boleni were 63.3 and 54.4 infected acini respectively. The implications of these results are important as they illustrate that the stock of tick for the transmission of a *T. parva* stock in the laboratory should be carefully selected. The indications are that the tick population plays a role in selecting the parasite population in the field and it was observed that *R. appendiculatus* Muguga, was the most efficient vector for this parasite. The nature of the tick population in an area could play a large role in epidemiology of theileriosis.

4.2 INTRODUCTION

The distribution of *Rhipicephalus appendiculatus*, the main field vector of *Theileria parva*, stretches from southern Sudan to Natal Province in South Africa (Norval *et al.*, 1992a). A closely related tick species *Rhipicephalus zambeziensis* which is also a field vector of *T. parva* is distributed in more limited areas of southern and central Africa. It can survive in drier areas than *R. appendiculatus* (Norval *et al.* 1992a). It is not surprising that biological differences occur between populations of *R. appendiculatus* in different geographical areas and also between *R. appendiculatus* and *R. zambeziensis*. Young *et al.* (in preparation b) reported that the populations of *R. appendiculatus* in southern Africa are larger than eastern African populations, which results in the development interval of *R. appendiculatus* instars being longer in southern and central African ticks than the eastern African ticks. While southern and central African populations of *R. appendiculatus* can undergo behavioural diapause in the adult instar, which results in only one generation of ticks occurring per year in these areas (Short & Norval, 1981; Pegram & Banda, 1990; Mazhawu, Mutapi, Marstand, Rusiwa, Berkvens, Norval, Pegram & Young, in press), eastern African tick populations do not show diapausing behaviour and can undergo two or more generations per year (Kaiser, Sutherst & Bourne, 1982; Young *et al.*, in preparation b). This results in a seasonal occurrence of instars of *R. appendiculatus* infesting hosts in southern and central Africa unlike in eastern Africa and it is this that generates different patterns of theileriosis occurrence in both areas (Koch, 1990; Moll *et al.*, 1986; Medley *et al.*, 1993a).

Because of the difference in behaviour and development of populations of rhipicephalid ticks and in the patterns of *T. parva* transmission in eastern, central and

southern Africa, it would be of importance to investigate the transmission competence of various rhipicephalid tick stocks from the different geographic regions for the transmission of *T. parva* stocks. This has not been studied previously as different stocks of rhipicephalid vectors of *T. parva* have not been generally available in any laboratory. A relevant study has been that of Kubasu (1992) where he compared the transmission competence of 5 stocks of *R. appendiculatus* from Kenya to transmit *T. parva* and found only minor differences in the competence of the strains. Also Lawrence *et al.*, (1983) and Blouin & Stoltsz (1989) compared the competence of *R. appendiculatus* and *R. zambeziensis* to transmit *T. parva* and *T. taurotragi* from cattle and buffalo. Both of these latter studies indicated that *R. zambeziensis* was a more competent vector of *T. parva* than *R. appendiculatus.*

In this study, we have compared the competence of *R. appendiculatus* and *R. zambeziensis* stocks from Kenya, Zambia and Zimbabwe to transmit *T. parva* stocks from Kenya and Zimbabwe to determine whether there are significant differences in transmission which could have important epidemiological effects.

4.3 MATERIALS AND METHODS

4.3.1 Comparative competence of rhipicephalid tick stocks for the transmission of *Theileria parva* Muguga

Three Boran steers (BJ126, BJ182 and BJ227), were inoculated with *T. parva* Muguga stabilate as described in Section 2.4.1. Two thousand uninfected nymphae of RAM, KEN KIA, ZIM WM, McILW, ZAM EP, ZAM SP and R. ZAM rhipicephalid tick stocks (Section 2.2) were applied to the cattle in separate cloth patches and allowed to feed to repletion (Section 2.4.1). After moulting, a batch of each tick stock that dropped replete from each steer was assessed for infection in their salivary glands using Feulgen's staining (Section 2.5).

This experiment was repeated using 4 Boran steers, numbered BL45, BL62, BL64 and BL66, but the second rather than the first generation of field ticks (Section 2.2) were applied. Four tick batches of each stock feeding to repletion on four consecutive days on each of the four steers were assessed for infection after moulting.

4.3.2 Comparative competence of rhipicephalid tick stocks for the transmission of *Theileria parva* Boleni

In a third experiment Freisian dairy breed cattle, numbered BK258 and BK314, were infected with 4 ml and 1 ml respectively of *T. parva* Boleni stabilate as described in Section 2.4.2. Two thousand uninfected nymphae of RAM, KEN KIA, ZIM WM, McILW, ZAM EP, ZAM SP and R. ZAM rhipicephalid tick stocks (Section 2.2)

were applied to the cattle and allowed to feed to repletion (Section 2.4). After moulting, four batches of each tick stock that fed to repletion on four consecutive days on each of the two steers were assessed for *T. parva* infection in their salivary glands using Feulgen's stain (Section 2.5).

4.4 RESULTS

4.4.1 Competence of transmission of *Theileria parva* Muguga by stocks of rhipicephalid ticks

When resultant infections of the tick batches in the two experiments were compared, *Rhipicephalus appendiculatus* Muguga had the highest mean abundance of infection in both experiments followed, in ranking order, by *R. appendiculatus* McIlwaine, *R. appendiculatus* ZAM EP and *R. appendiculatus* KEN KIA (Figure 4.1). The ranking order for the prevalence of infection of the first four tick stocks was the same as that of abundance of infection in both experiments. The overall ranking of vector competence, abundance of infection being the determinant, was 1) *R. appendiculatus* Muguga, 2) *R. appendiculatus* McIlwaine, 3) *R. appendiculatus* ZAM EP, 4) *R. appendiculatus* KEN KIA, 5) *R. zambeziensis* Nuanetsi, 6) *R. appendiculatus* ZIM WM, 7) *R. appendiculatus* ZAM SP (Figure 4.2).

There was a difference of about 3.7 times in the level of mean abundance between the tick stocks, with the highest abundance of *T. parva* infection (*R. appendiculatus* Muguga), and tick stock with the least (*R. appendiculatus* ZAM SP). If the mean prevalence of infection was considered, it was found that the ranking order was similar to that obtained using the abundance of infection (Figure 4.2).

The pattern of prevalence and abundance in male and female ticks is shown in Table 4.1. The same pattern occurred in male and female tick infections for the 7 rhipicephalid stocks.

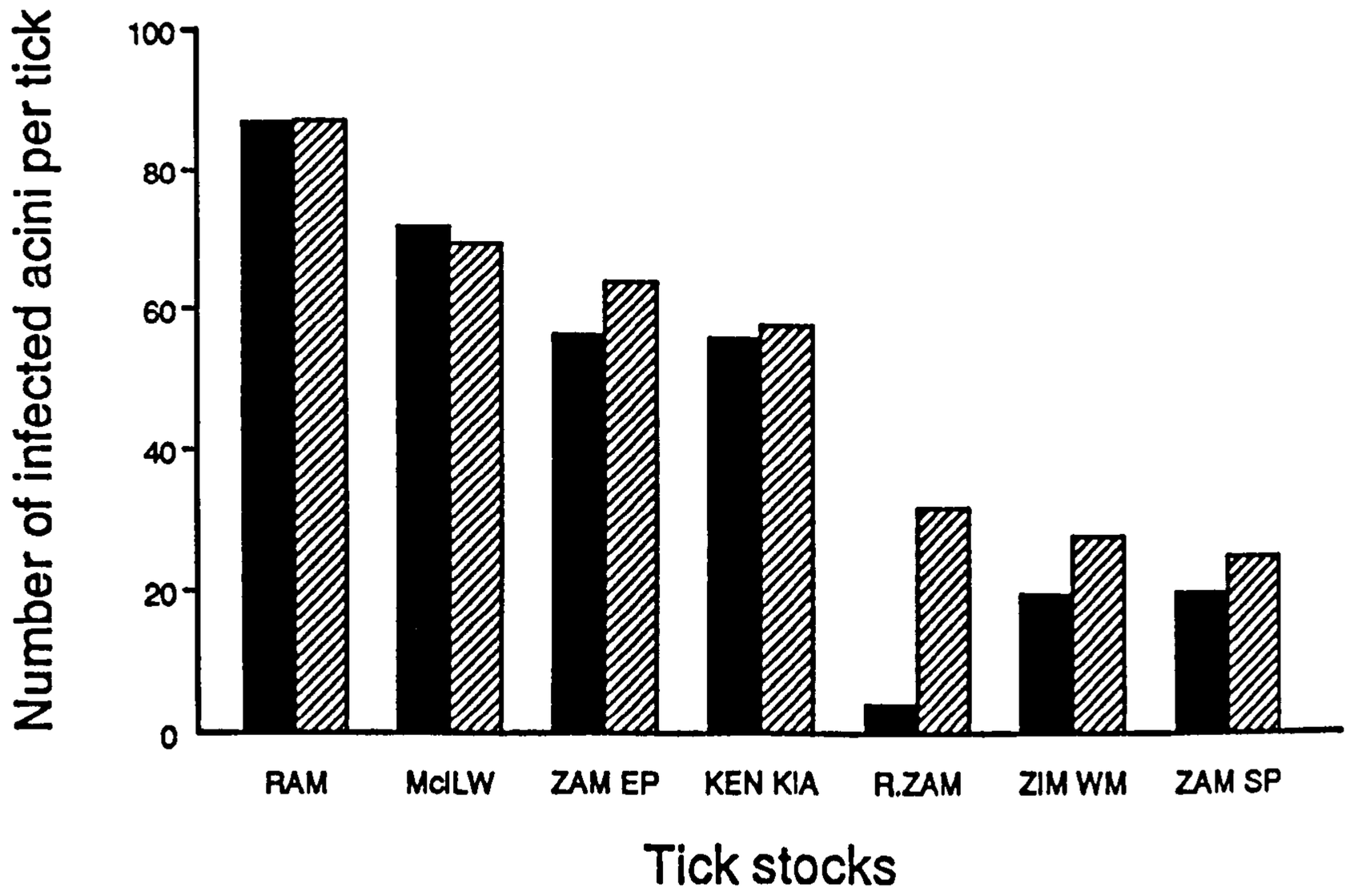


Figure 4.1 Abundance of infection developing in two groups of adult rhipicephalid ticks, each containing 7 different stocks, after feeding to repletion as nymphae on cattle suffering acute *Theileria parva* Muguga infections.

Results from the first group of ticks (■) were obtained from a total of 21 batches (a batch of each stock from each of the three bovine hosts). The second group (▨) contained results from a total of 112 tick batches (four batches of each tick stock feeding to repletion on each of four animals).

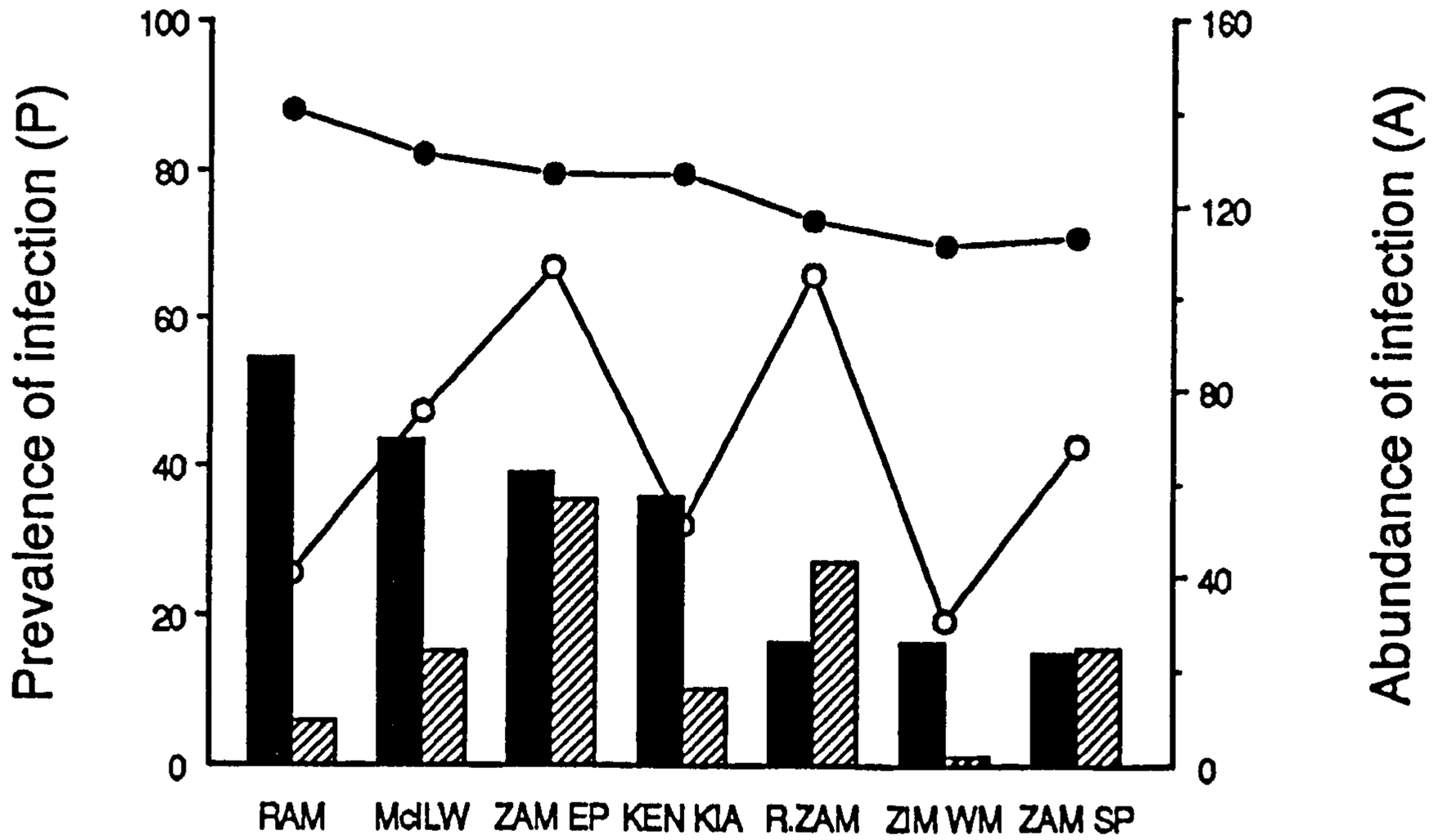


Figure 4.2 Prevalence and abundance of infections developing in different stocks of *Rhipicephalus appendiculatus* adults feeding to repletion as nymphae on cattle suffering acute *Theileria parva* Muguga (● = prevalence; ■ = abundance) or Boleni infections (O = prevalence; ▨ = abundance).

Fifty six tick batches, that is, 4 batches of each stock feeding to repletion on four consecutive days on each of two animals were assessed.

4.4.2 Competence of transmission of *Theileria parva* Boleni by stocks of rhipicephalid ticks

The abundance of *T. parva* Boleni infection developing in the different tick stocks is shown in Figure 4.2. On the basis of mean abundance of infection, the highest *T. parva* Boleni infection was found in *R. appendiculatus* ZAM EP and lowest in *R. appendiculatus* ZIM WM. There was a difference of about 28 times in mean abundance between these two stocks. *Rhipicephalus zambeziensis* proved to have the second highest mean abundance followed by *R. appendiculatus* ZAM SP, McIlwaine, Ken Kia and Muguga. If the prevalence of infection was compared for the tick stocks a similar pattern was seen (Figure 4.2) as was the pattern for infection in male and female ticks (Table 4.1).

Table 4.1 Mean prevalence and abundance of infection in rhipicephalid tick stocks infected with *Theileria parva* Muguga and Boleni stocks. The mean infection levels of the Muguga parasite stock were computed from two groups of ticks. The first group comprised 112 tick batches (four batches of each stock that fed to repletion on consecutive days on each of four cattle. The second group comprised 63 batches (three batches of each stock that fed to repletion on three cattle). The mean infection levels from both groups of ticks were computed. Fifty six tick batches, comprising of four batches of each stock that fed to repletion on each of two animals infected with *T. parva* Boleni parasite stock were assessed.

Tick gender	Tick stock	*Theileria parva* Muguga		*Theileria parva* Boleni	
		Prevalence of infection	Abundance of infection	Prevalence of infection	Abundance of infection
Male	RAM	84.2	46.2	19.6	4.1
	KEN KIA	71.8	28.6	23.8	11.4
	ZAM EP	74.4	35.2	58.8	25.7
	ZAM SP	59.3	9.5	39.2	17.5
	McILW	74.4	31.0	43.8	13.5
	ZIM WM	63.9	16.4	15.0	0.7
	R. ZAM	69.6	13.9	62.1	25.2
Female	RAM	91.9	127.9	31.7	14.7
	KEN KIA	87.2	85.6	40.0	20.8
	ZAM EP	84.4	89.9	74.6	87.3
	ZAM SP	75.4	37.9	45.8	32.2
	McILW	91.2	108.2	50.8	34.8
	ZIM WM	75.3	35.8	23.3	3.4
	R. ZAM	76.5	38.7	69.2	61.1
Both	RAM	88.1	87.1	25.6	9.4
	KEN KIA	79.4	57.1	31.9	16.1
	ZAM EP	79.5	62.5	66.7	56.5
	ZAM SP	70.9	23.8	42.5	24.9
	McILW	82.2	69.6	47.3	24.2
	ZIM WM	69.7	26.1	19.2	2.1
	R. ZAM	73.1	26.3	65.6	43.2

4.4.3 A comparison of competence of rhipicephalid ticks for the transmission of the Boleni and Muguga stocks of *Theileria parva*

The ranking of the comparative competence of the rhipicephalid stocks for the transmission of *T. parva* Boleni and Muguga is shown in Table 4.2 and Figure 4.2. On the basis of the mean abundance of infection, there is a different pattern of comparative vector competence for the two stocks of *T. parva* Boleni and Muguga. For example, whereas *R. appendiculatus* Muguga developed the highest abundance for *T. parva* Muguga, *R. appendiculatus* ZAM EP developed the highest abundance for *T. parva* Boleni. *Rhipicephalus appendiculatus* ZAM SP developed the lowest abundance for *T. parva* Muguga infection, while *R. appendiculatus* ZIM WM developed the lowest abundance for *T. parva* Boleni infection.

When the results were analysed statistically (Chapter 6), there was a significant difference between the Muguga and Boleni stocks of *T. parva* infections established in the different tick stocks ($P < 0.01$). Likewise, there were significant differences within each parasite stock ($P < 0.05$) developing between the tick stocks.

Table 4.2 The comparative competence of rhipicephalid tick stocks for the transmission of *Theileria parva* Muguga and Boleni stocks. The mean infection levels of the former stock were computed as described in Table 4.1.

Tick stock	*Theileria parva* Muguga		*Theileria parva* Boleni	
	Ranking for vector competence	Proportion of mean abundance of *T. parva* compared to the least efficient vector	Ranking for vector competence	Proportion of mean abundance of *T. parva* compared to the least efficient vector
RAM	1	3.66	6	4.48
KEN KIA	4	2.40	5	7.67
ZAM EP	3	2.63	1	26.90
ZAM SP	7	1.00	3	11.86
McILW	2	2.92	4	11.52
ZIM WM	6	1.10	7	1.00
R. ZAM	5	1.14	2	20.57

4.5 DISCUSSION

In this study it was shown that laboratory populations of *R. appendiculatus* and *R. zambeziensis* established from tick populations in Kenya, Zambia and Zimbabwe had different degrees of susceptibility to two stocks of *T. parva*, one from Kenya and other from Zimbabwe. These differences in susceptibility were shown to be significant and reproducible and it is probable that they suggest differences in the ability to transmit the parasite even though this was not tested directly. Three main features of these findings to be discussed are the experimental use, the mechanism(s) by which this phenomenon occurs and the epidemiological significance.

It is evident from these results that different combinations of stocks of *T. parva* and rhipicephalid ticks affect the levels of infection in the vector, and likely therefore the transmission competence. For example, while *R. appendiculatus* Muguga was shown to be the efficient vector of *T. parva* Muguga out of the seven stocks investigated, it was not the most efficient vector for *T. parva* Boleni. This is not surprising, since over the last 40 years, *T. parva* Muguga has been maintained in the laboratory by transmission with *R. appendiculatus* Muguga (Bailey, 1960). Therefore it could be expected that the *T. parva* Muguga parasite population and *R. appendiculatus* Muguga have co-adapted following a long-term laboratory relationship between the parasite and the tick stock. For the Boleni stock of *T. parva*, the most efficient vector was found to be *R. appendiculatus* ZAM EP (established from the field population in 1992) and not *R. appendiculatus* McIlwaine, the long-term laboratory stock used in Zimbabwe.

For experimental use this study demonstrates that much higher infections of *T. parva* can develop in carefully chosen tick stocks. The most striking example is with *R. appendiculatus* ZIM WM stock where approximately 28 times lower abundance of *T. parva* Boleni infection was obtained than for the *R. appendiculatus* ZAM EP stock. Hence if the objective was to transmit acute infections in the laboratory or to harvest sporozoites in bulk from ticks, it would be essential to use the *R. appendiculatus* ZAM EP stock rather than the ZIM WM stock for the transmission of *T. parva* Boleni. As far as experimental transmission in Kenya is concerned, the most competent vector stock, *R. appendiculatus* Muguga, is already being used for transmission of *T. parva* Muguga (Young *et al.* in preparation a).

Following this argument, there would appear to be a possibility that the *T. parva* parasite is selected by the tick stock prevailing in an area and not *vice versa* since *T. parva* infection levels in field ticks are never high enough to be detrimental to the survival of tick populations (Walker, Young & Leitch, 1981; Young *et al.*, 1986; Chapter 5). Hence it is only parasite populations which complete their life cycle in the ticks which are going to be efficiently maintained in the field. Although it is recognized that overdispersion occurs in tick populations infected with *T. parva* (Büscher & Otim, 1986; Young *et al,.* in preparation a; Chapter 6), there is some controversy as to whether overdispersion of *T. parva* infection occurs by chance or is the product of more favourable conditions occurring within individual ticks. The evidence from this study is that conditions within the tick are important for development of infection. Hence within the same country (Zimbabwe), the McIlwaine stock of *R. appendiculatus* appears to provide a much more favourable conditions for development of *T. parva* parasites than the stock from Mashonaland West. Using such stocks of ticks, it may be possible to locate the mechanism(s)

involved in the degree of susceptibility of tick stocks for *T. parva* infections. A simple approach would be to compare the development of sexual stages in tick stocks of lower and higher susceptibility. It is possible that the level of proteases and lectins in the gut of the engorged nymphae may have a marked effect on development of the sexual stages (Mehlhorn & Schein, 1984; Mehlhorn & Schein, 1993; Shaw & Young, 1994) as has been demonstrated for the development of trypanosomes in tsetse flies (Welburn, Maudlin & Ellis, 1989; Stiles, Ingram, Wallbanks, Molyneux, Maudlin & Welburn, 1990; Maudlin & Welburn, 1987; Mihok, Otieno, Darji & Munyinyi, 1993). This could be best investigated using the *in vitro* feeding technique developed by Waladde, Young, Ochieng', Mwaura and Mwakima (1993). In Chapter 3, it has been indicated that differences in the numbers of the target cells ("e" cells of type III acini) in the salivary glands for *T. parva* development differs between different instars which would affect *T. parva* infection levels. The number of target cells could also vary between *R. appendiculatus* stocks but this was not examined.

It must be stressed that these results on vector competence were obtained under laboratory conditions which must be considered artificial. Nevertheless, as the differences in vector competence for *T. parva* stocks were great they would also be expected to apply under field conditions. For example, stocks of *T. parva* in Zimbabwe appear under field conditions to be less virulent than stocks in East Africa (Koch, 1990; Irvin *et al.*, 1989). This could be explained by the lower susceptibility of the field stock of *R. appendiculatus* to *T. parva* in Zimbabwe than in eastern Africa. In contrast, the Zambian stock of *R. appendiculatus* (ZAM EP), which appears to be an efficient vector of *T. parva*, occurs in a region where East Coast fever of a virulent type is prevalent. It is difficult to envisage how this feature could

be investigated further to obtain conditions similar to those occurring in nature. As Medley *et al.* (1993a) and Chapter 4 have shown that in *T. parva* infection, carrier cattle are very important in the maintenance of infected ticks, a comparison of the competence of different *R. appendiculatus* stocks to become infected when fed on *T. parva* carrier animal may be a more realistic method to investigate this phenomenon.

CHAPTER 5

SURVIVAL OF *THEILERIA PARVA* IN INSTARS OF ITS TICK VECTOR *RHIPICEPHALUS APPENDICULATUS* UNDER DIFFERENT CONDITIONS.

5.1 SUMMARY

Groups of nymphal and adult *Rhipicephalus appendiculatus* Muguga, having different intensities of *Theileria parva* Muguga infection in their salivary glands, were exposed to various temperature and humidity conditions in the laboratory and quasi-natural climatic conditions. Survival of the ticks and *T. parva* infections in their salivary glands was determined over time. In adult ticks having an abundance of infection of 26 infected acini per female, the shortest survival time of the ticks occurred under a diurnal temperature rhythm of 23-30°C, 85% RH. This temperature rhythm represented that at the coast of Kenya. The longest survival in the laboratory of the same group of ticks and their infection was obtained under a diurnal temperature rhythm of 13-23°C, representing the temperature range at Kabete, Kenya. The survival curve of these ticks at a diurnal temperature rhythm of 13-23°C was similar to that obtained under natural conditions; it took 58 weeks of exposure to reach 50% survival compared to 63 weeks under natural conditions. In contrast, the *T. parva* parasites survived for much longer under quasi-natural climatic conditions than under any of the laboratory conditions tested. Under quasi-natural climatic conditions, the parasite survived as long as the tick while it fell dramatically between weeks 10 and 18 after exposure to all the laboratory conditions tested. Similar results were obtained when the survival of ticks having a lower intensity of infection, that is, an abundance of 2 infected acini per female tick, was compared. However there was apparently a density dependent relationship with no dramatic fall in infection occurring in the adult ticks with a lower density of infection (2 infected acini per female tick). When nymphal ticks having an abundance of infection of 2 infected acini were studied, it was found that they survived for 66 weeks under quasi-natural climatic conditions compared to 102 weeks for adult ticks

of similar infections under the same conditions. The period to 50% survival of the nymphae was 48 weeks. However, the parasite survival curve for the nymphs was much shorter than for adults. There was no dramatic decline in infections in nymphae with lower infections (2 infected acini per nymph) exposed to quasi-natural climatic conditions. At 20°C in the laboratory, the parasite survival curves in the nymphal ticks were similar to those of the parasites in the adult ticks exposed to the same conditions.

5.2 INTRODUCTION

Little progress on the understanding of the effect of climate and environment on the development and survival of *T. parva* within its tick vector under natural conditions has been made since Theiler (1905b) reported that *T. parva* infections in paddocks in South Africa would die out within 15 months. This is despite the fact that temperature is known to have a large effect on the development of *T. parva* in the tick and on the tick itself (Young & Leitch, 1981). There have been several laboratory studies on the survival of *T. parva* in the tick (Lewis & Fotheringham, 1941; Lewis, 1950; Martin, Barnett & Vidler, 1964) which have demonstrated that the parasite may survive for about 12 months in the salivary glands of adult ticks, a much shorter period than the adult ticks themselves under these conditions. Even though environmental conditions are known to vary considerably throughout the range of *T. parva* (Dallwitz *et al.* 1986; Perry *et al.*, 1990a), with most areas having marked diurnal temperature fluctuations, most information has been obtained on the development of *Theileria* parasites in the nymphal/adult ticks under constant temperature conditions and no consideration of the larval/nymphal development. The only published reports on the longevity of ticks exposed to field conditions are from Kenya at altitudes of 1600 and 2100m (Young, Leitch, Dolan, Newson, Ngumi & Omwoyo, 1983b; Newson, Chiera, Young, Dolan, Cunningham & Radley, 1984; Young, Leitch, Morzaria, Irvin, Omwoyo & DeCastro, 1987). In these studies it was shown that *T. parva* survived in adult ticks for at least 19 months at 2100m altitude and for 15 months at 1600m altitude. More accurate and extensive information on the survival of the tick and the *T. parva* parasite under laboratory and quasi-natural climatic conditions would be useful for the control of the parasite and for modelling its transmission. Ultimately, the models would also be used to help in

the design of viable theileriosis control methods. For experimental use, it is important to know how long high infections survive in the tick so that ticks can be utilized during this time to obtain large numbers of *T. parva* sporozoites.

In this study, adult and nymphal *R. appendiculatus* ticks infected with *T. parva* were exposed to different climatic conditions in the laboratory and under quasi-natural field conditions at 1950m altitude in Kenya to obtain survival curves for both parasites and ticks. Adult and nymphal ticks with high, medium and low intensity of *T. parva* infections were also used in these experiments to determine whether the density of *T. parva* infection also had an effect on parasite survival.

5.3 MATERIALS AND METHODS

5.3.1 Exposure of ticks to quasi-natural conditions

Nymphal and adult ticks to be exposed to natural climatic conditions were introduced into tubes, 50cm long by 4cm in diameter, made from nylon bolting silk material of 400μm mesh (Nybolt, Swiss Silk Bolting Cloth Manufacturing Co. Ltd., Zurich, Switzerland). The tubes were sealed at the top and kept upright in grass using thin, steel wire supports in a cage. The cage, enclosed in chicken wire and containing naturally growing vegetation, was located adjacent to the Tick Unit, ILRAD, Nairobi. The climatic conditions in the field were recorded at the adjacent Kabete Meteorological Station.

5.3.2 Decline of *Theileria parva* infection in salivary glands and survival of the highly infected adult tick vector

Approximately six tubes containing uninfected nymphal *R. appendiculatus* Muguga were applied on alternate days in cloth bags glued to shaved areas on the back of each of four Boran steers, numbered BJ110, BJ131, BJ135 and BJ136, parasitaemic for *T. parva* Muguga infection. The ticks were allowed to feed to repletion (Section 2.4.1).

Nymphs dropping replete from animal BJ135 on 20-28th May, 1992, (day 15 to 23 post infection) when the animal had a piroplasm parasitaemia of between 3.1 and 19.2%, were selected. The engorged nymphs were pooled, as the number of ticks required for the experiment was large (24,480), mixed thoroughly and then

redistributed equally into tick tubes before being kept to moult at 24°C and approximately 85% RH for 28 days. Because of the necessity to pool the ticks, the median date of repletion (24th May, 1992) became the representative date of repletion for the group. The resultant adult ticks from these nymphae were categorized as having "high" infections (See Table 5.2 for their average abundance of infection).

On day 28 post-repletion, 30 female ticks were dissected and their salivary glands examined for the presence of the *T. parva* Muguga parasite using the Polymerase Chain Reaction (PCR) technique (Bishop *et al.* 1992). As a control group, 30 uninfected ticks were also dissected and their salivary glands examined for *T. parva* Muguga by PCR concurrently. As positive PCR reactions were detected on this day, it was assumed that this was the first day of salivary gland infection. This was denoted "day 0 salivary gland infection". The ticks were redistributed at 240 per tube and groups were maintained under the following conditions:

Group A (24 x 240 ticks) - 20°C, 85% RH

Group B (12 x 240 ticks) - 24°C, 85% RH

Group C (24 x 240 ticks) - programmed incubator set for a diurnal temperature rhythm of 13-23°C and 85% RH.

Group D (18 x 240 ticks) - programmed incubator set for a diurnal temperature rhythm of 23-30°C and 85% RH.

Group E (24 x 240 ticks) - kept in the field at ILRAD (Section 5.3.1)

Programmable cooled incubators with timed cycling of temperature and illumination, were used in the laboratory (Gallenkamp Cooled Incubator Inf-781-T, Sanyo

Gallenkamp Plc., Leicestershire, England). These allowed for the setting of diurnal temperature-light combinations.

On day 14 post salivary gland infection, 30 male and 30 female ticks from each group were fed on rabbits for 4 days then assessed for salivary gland infections after Feulgen's staining (Section 2.5). Assessment of the ticks for infections was repeated at monthly intervals thereafter and the number of male and female ticks surviving recorded at the same time.

5.3.3 Decay of *Theileria parva* infection and survival of adult ticks having medium and low infections

Thirty male and 30 female ticks from different *T. parva* Muguga-infected batches were fed on rabbits for 4 days then assessed for infections using Feulgen's stain (Section 2.5) to select two categories of ticks with different infection levels.

Ticks dropping replete from animal BJ170 on 12th, 13th and 15th July, 1992 (day 21, 22 and 24 respectively post infection) were selected for use in this experiment. The tick batches of 12th and 13th July, 1992 had an average infection level of about 780 infected acini per 30 females, equivalent to an average of about 26 infected acini per female tick. These batches were pooled to obtain enough ticks for this infection level category, which for convenience, was referred to as the "medium" infection category. The tick batch dropping replete on 15th July, 1992 had an average infection of about two infected acini per female tick and they were used for the second infection level category, referred to as the "low" infection category. Day 0 of salivary gland infection was considered to be day 28 after repletion of the nymphs

as this duration had been demonstrated for the same parasite in the same tick stock (Section 5.3.2).

At week 10 post-repletion, when the ticks had been exposed to 24°C for 4 weeks and 20°C for 6 weeks, the ticks from each category were exposed to either 20°C, 85% RH or field conditions (Section 5.3.1) as follows:-

20°C, 85% RH - 14 x 200 ticks of medium infections

Field - 18 x 200 ticks of medium infections

20°C, 85% RH - 14 x 200 ticks of low infections

Field - 18 x 200 ticks of low infections.

The ticks were assessed at monthly intervals and the number of surviving ticks recorded at the same time.

5.3.4 Decay of *Theileria parva* infection and survival of the highly infected nymphal vector

Two tubes containing uninfected larval *R. appendiculatus* were applied on alternate days on the ears of each of four Boran steers, numbered BJ126, BJ182, BJ213 and BJ227, parasitaemic for *T. parva* Muguga infection, and allowed to feed to repletion (Section 2.4.1).

Larvae dropping replete from animal BJ 182 on 8th, 9th and 10th August, 1992 (day 17, 18 and 19 respectively post infection), when it had a piroplasm parasitaemia of 25.6, 34.3 and 60.2% respectively were pooled for use in this experiment. Thus the

median date of 9th August, 1992 was taken as the representative day of repletion for the group. The replete larvae were kept at 24°C and approximately 85% RH to moult. For convenience, the resultant nymphae were categorized as having "high" infections. On day 21 post-repletion, 30 resultant nymphae were dissected, their salivary glands dabbed on glass slides then stained in Giemsa. These were subsequently examined under oil emersion for the presence of *Theileria* sporoblasts. Sporoblasts were detected on day 21 post-repletion and thus denoted "day zero salivary gland infection". On this day, the nymphae were divided into two groups, one being kept at 20°C, 85% and the other kept in the field at ILRAD (Section 5.3.1).

On day 14 after salivary gland infection was detected, 60 nymphae from each group were fed on rabbits for 3 days then assessed routinely for salivary gland infection after Feulgen's staining. Assessment of the infections was repeated at monthly intervals thereafter and the number of nymphs still alive counted at the same time.

5.3.5 Decay of *Theileria parva* and survival of the lowly infected nymphal vector

Larvae were routinely infected with *T. parva* Muguga on several Boran steers as described in Section 2.4.1. The replete ticks were then kept at 24°C and approximately 85% RH for 28 days to moult. The resultant nymphae were transferred to 20°C and 85% RH for 14 days to harden then assessed for salivary gland infection using Feulgen's stain.

The nymphal tick batch dropping replete from animal BJ435 on 14th April, 1993 was selected for use in this experiment. They had an average of about 2 infected acini each and were referred to as having "low" infections. They were divided into groups of 200, one half of which were kept at 20°C, 85% RH and the other half in the field (Section 5.3.1). The ticks were assessed for *T. parva* salivary gland infection at monthly intervals and the number of nymphs still alive counted and recorded at the same time.

5.4 RESULTS

Categorization of adult ticks as having "high" infections is described in Section 5.3.2 and "medium" or "low" infections in Section 5.3.3. Categorization of "high" or "low" infections in nymphal ticks is given in Section 5.3.4 and 5.3.5 respectively.

5.4.1 Climatic conditions during the period of exposure of ticks to natural conditions

The climatic conditions during the period of exposure of the ticks to quasi-natural conditions is shown in Figure 5.1. In the period June to December 1992, the mean monthly maximum temperatures varied from 19 to 26°C and the mean monthly minimum temperatures from 10 to 14°C. The mean monthly relative humidity at 1500h varied from about 40 to 70% RH while the total monthly rainfall varied from 15 mm to 215 mm.

Typically, Kenya experiences one long rainy season around February to June and a short rainy season in August to October. Usually, July is the coldest month while January is the hottest and driest month. However, the first half of 1993 was quite atypical. For example, the month of January was the wettest and also not the hottest. The total amount of rainfall during the long rainy season was far below the usual average. The second half of the experimentation period (July, 1993 to June, 1994) was more typical however. Exposure to field conditions of the highly infected adult ticks and the lowly infected nymphae commenced in June, 1992. Adult ticks having medium and low infections and nymphae having high infections were exposed to field conditions as from September, 1992.

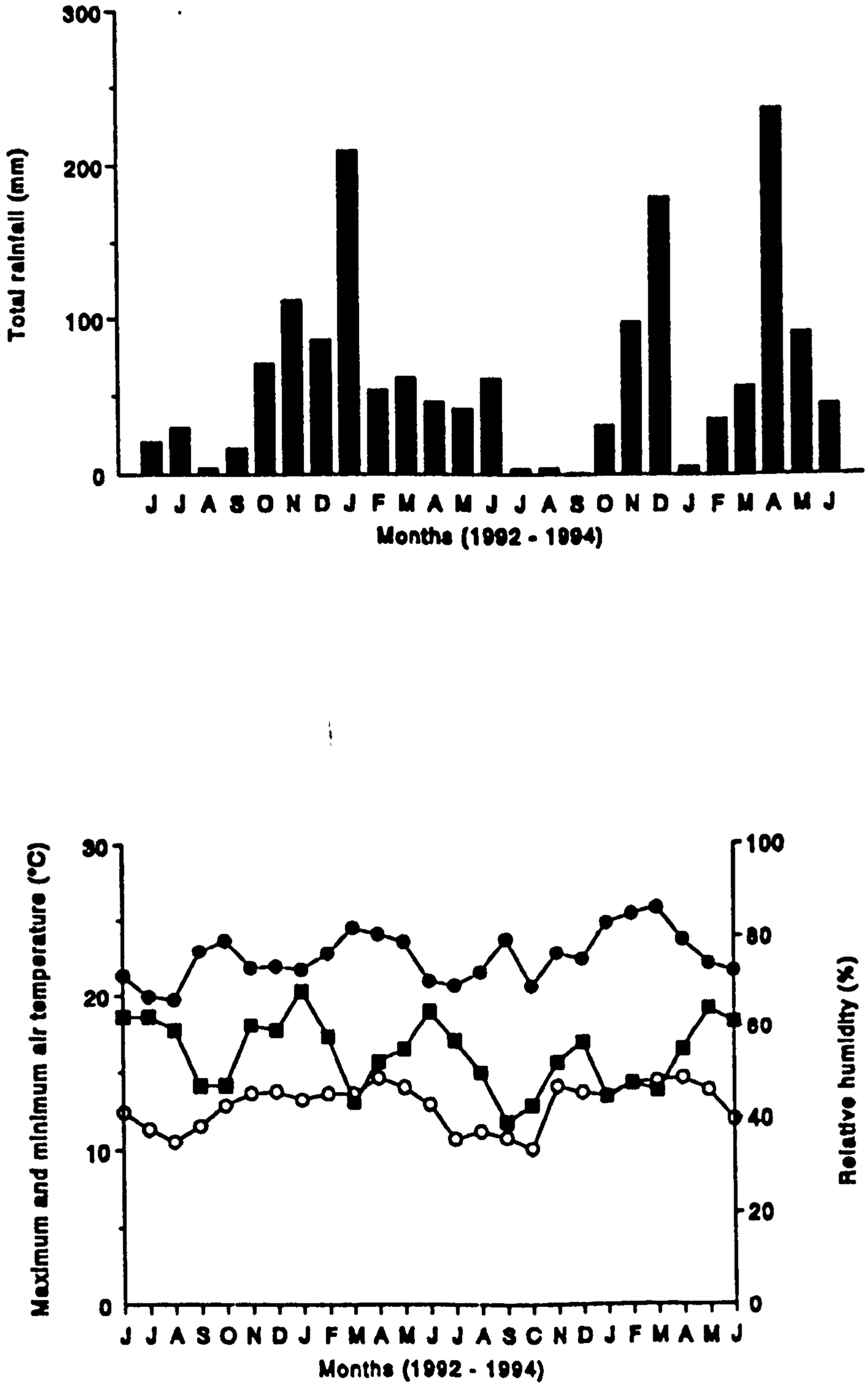


Figure 5.1. Total monthly rainfall (■), mean maximum (●) and mean minimum (O) monthly air temperatures and relative humidity at 1500 H (▲) recorded at the Kabete Meteorological Station, Kabete, Kenya from June 1992 to June 1994 during the period of exposure of ticks to quasi natural climatic conditions.

5.4.2 Survival of *Theileria parva* in "highly" infected adult *Rhipicephalus appendiculatus*

Survival curves for *T. parva* infection in the salivary glands of the "highly" infected ticks are shown in Figure 5.2. Ticks exposed to natural conditions had a longer survival of *T. parva* in their salivary glands than in any ticks kept under laboratory conditions. The survival curves from laboratory ticks were very similar to each other and did not seem to be dependent on the temperatures to which they were exposed to. The second most suitable condition for the duration of *T. parva* survival was at 13-23°C, 85% RH while the other 3 conditions (20°C, 24°C, 23-30°C, all at 85% RH) had virtually identical survival curves. When the survival of the parasites under the five conditions were analysed statistically(Chapter 6), there was no significant difference between the four different laboratory conditions ($p > 0.05$). However the ticks kept under field conditions survived for a significantly longer time than those kept under any of the 4 laboratory conditions ($p < 0.01$). The adult tick groups were analysed for the survival of *T. parva* infection in their salivary glands for 50% and 100% mortalities and the results are shown in Table 5.1.

If the survival of *T. parva* parasites in salivary glands of male and female ticks was considered separately, similar patterns of survival were found in the male and female tick groups (Table 5.2). Infection levels were higher in the female ticks than in the male ticks, which is normal, but tick gender did not appear to influence survival of the parasite.

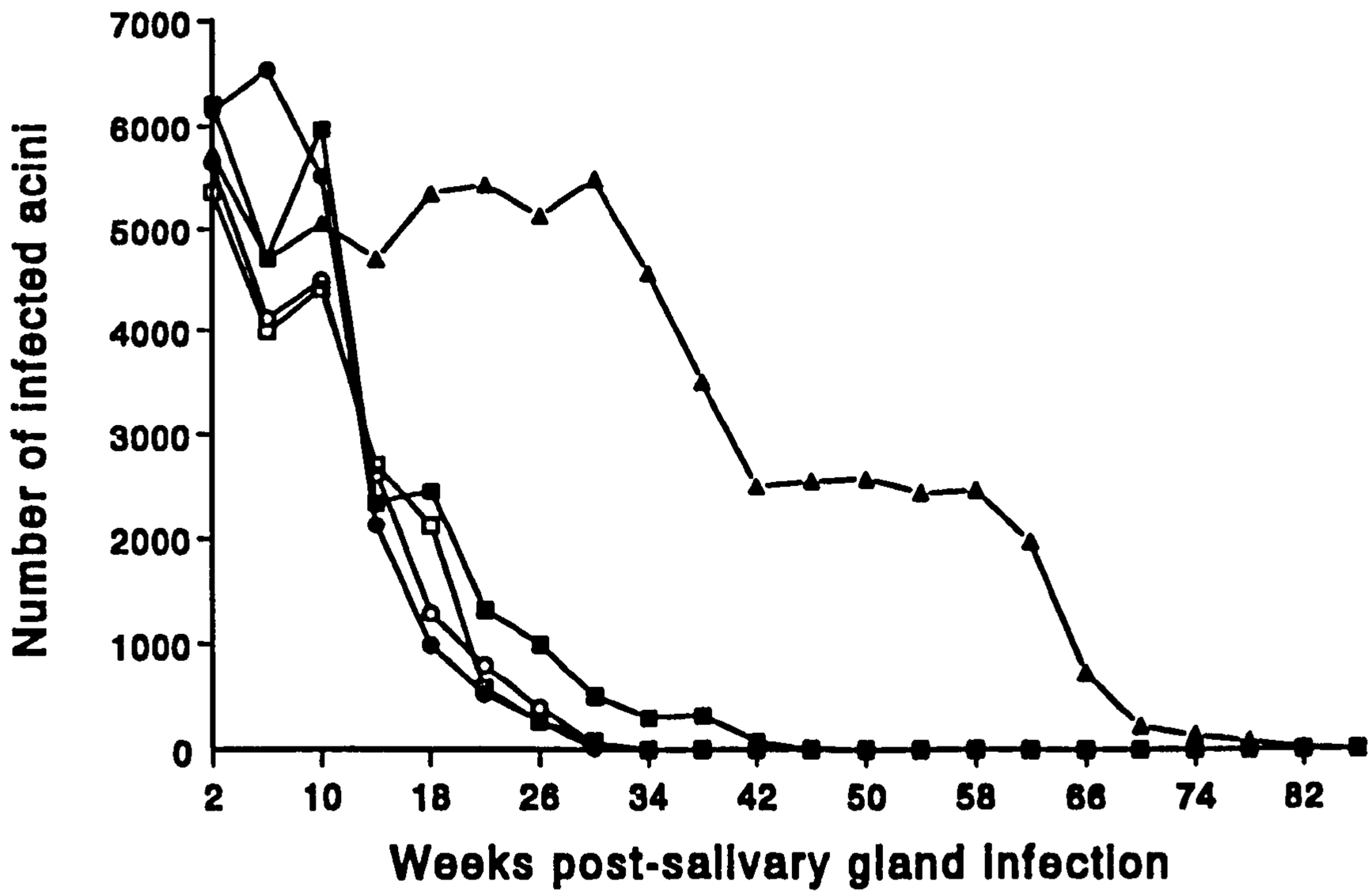


Figure 5.2. Survival of *Theileria parva* Muguga in the highly infected adult *Rhipicephalus appendiculatus* Muguga after exposure to different climatic conditions.

Table 5.1. Period in weeks to the fifty and hundred percent mortality of *Theileria parva* in the salivary glands of highly infected *Rhipicephalus appendiculatus* adults and nymphae under different conditions. All ticks kept in the laboratory were at 85% RH.

Instar	Conditions	Period to 50% mortality of *T. parva* (weeks)	Period to 100% mortality of *T. parva* (weeks)
Adults	Constant 20°C	13	28
	Constant 24°C	13	32
	Diurnal rhythm 13-23°C	13	44
	Diurnal rhythm 23-30°C	13	32
	Quasi-natural conditions	41	78
Nymphae	Constant 20°C	13	26
	Quasi-natural conditions	27	50

Table 5.2. Survival of *Theileria parva* Muguga in the highly infected adult *Rhipicephalus appendiculatus* Muguga after exposure to different laboratory and quasi-natural conditions.

Tick gender	Conditions of exposure	Weeks after infection of salivary glands										
		2	6	10	14	18	22	26	30	34	38	42
Females	20°C	4567	4355	3916	1521	497	492	250	2	0	0	0
	24°C	3635	2480	1938	1831	721	344	166	12	0	0	0
	13-23°C	4727	3141	3106	1700	1892	800	501	102	61	25	12
	23-30°C	3825	3059	3079	1775	1239	265	57	7	0	0	0
	FIELD	4084	3472	3294	3698	3148	3138	3279	3608	3384	2543	1455
Males	20°C	1581	2181	1594	600	495	38	24	17	1	0	0
	24°C	2009	1636	2545	777	564	450	230	46	0	0	0
	13-23°C	1482	1576	2854	637	555	523	497	409	245	67	61
	23-30°C	1533	925	1320	926	880	313	214	67	3	0	0
	FIELD	1648	1235	1754	1001	2193	2289	1843	1865	1169	944	1041
Both	20°C	6148	6536	5510	2121	992	530	274	19	1	0	0
	24°C	5644	4116	4483	2608	1285	794	396	58	0	0	0
	13-23°C	6209	4717	5960	2337	2447	1323	998	511	306	320	73
	23-30°C	5358	3984	4399	2701	2119	578	271	74	3	0	0
	FIELD	5732	4707	5048	4699	5341	5427	5122	5473	4553	3487	2496

Table 5.2 (contd.).

Tick gender	Conditions of exposure	Weeks after exposure of ticks to different conditions										
		46	50	54	58	62	66	70	74	78	82	86
Females	20°C	0	0	0	0	0	0	0	0	0	0	0
	24°C	0	0	0	0	0	0	0	0	0	0	0
	13-23°C	0	0	0	0	0	0	0	0	0	0	0
	23-30°C	0	0	0	0	0	0	0	0	0	0	0
	FIELD	1318	1303	1250	1216	1062	368	102	63	42	10	0
Males	20°C	0	0	0	0	0	0	0	0	0	0	0
	24°C	0	0	0	0	0	0	0	0	0	0	0
	13-23°C	10	0	0	0	0	0	0	0	0	0	0
	23-30°C	0	0	0	0	0	0	0	0	0	0	0
	FIELD	1229	1269	1190	1249	903	365	115	70	39	11	0
Both	20°C	0	0	0	0	0	0	0	0	0	0	0
	24°C	0	0	0	0	0	0	0	0	0	0	0
	13-23°C	10	0	0	0	0	0	0	0	0	0	0
	23-30°C	0	0	0	0	0	0	0	0	0	0	0
	FIELD	2547	2572	2440	2465	1965	733	217	133	81	21	0

5.4.3 Survival of *Theileria parva* in adult *Rhipicephalus appendiculatus* which had medium and low infections

Two additional groups of *R. appendiculatus* adults having medium and low infections of *T. parva* were observed for the survival of *T. parva* in their salivary glands (Figure 5.3). Infection levels over time in ticks having medium infections and kept in the laboratory at 20°C, 85% RH had a similar pattern to that in ticks with high infections. In both cases, the infection levels plummeted from week 14 to 22 after salivary gland infection and finally died out by week 38. In contrast, ticks kept under quasi-natural climatic conditions showed a fairly consistent infection intensity until week 38. After that time, the infection levels decreased until infection died out by week 78. Ticks with low levels of infection showed a less dramatic decrease in infection intensity when kept at 20°C, 85% RH (Figure 5.3), however infections still died out by week 38. In contrast, the ticks kept under quasi-natural climatic conditions showed no marked decrease of infection up to week 62 after infection, but after that time, the infection decreased and died out by week 78. As was the case with the high infections, there was also a statistically significant difference between the parasites kept in the field and at 20°C, 85% RH in both cases ($p < 0.01$). When intensity of *T. parva* infections in male and female ticks were compared, the survival of infection in the two sexes showed a similar pattern (Table 5.3).

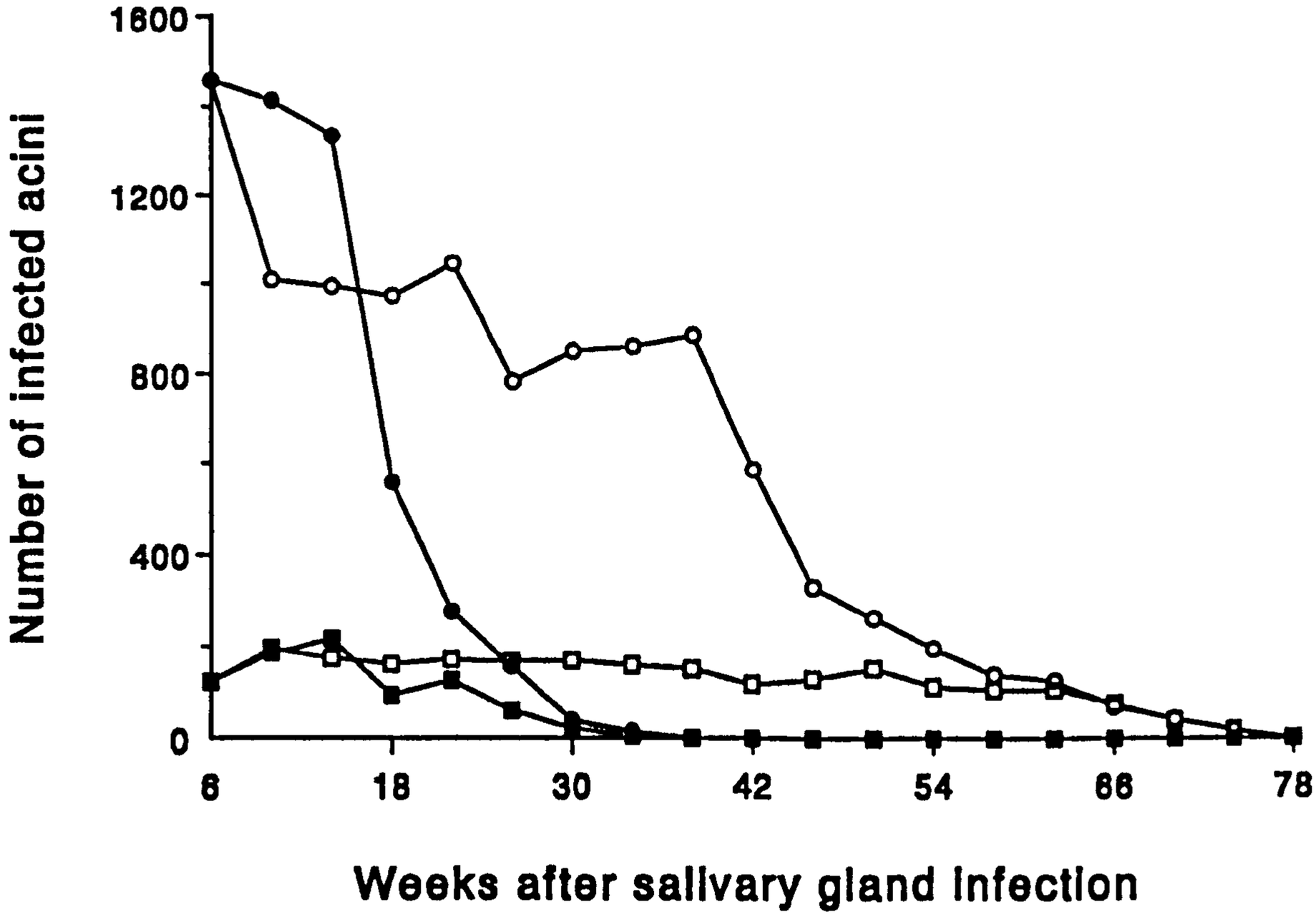


Figure 5.3. Survival of *Theileria parva* Muguga in adult *Rhipicephalus appendiculatus* Muguga having medium and low infections.

Table 5.3. Survival of *Theileria parva* Muguga in adult male and female *Rhipicephalus appendiculatus* Muguga having medium and low infections. M = adult male ticks; F = adult female ticks.

Weeks after salivary gland infection	Gender of tick	Total number of infected acini			
		Medium infections		Low infections	
		20°C	Field	20°C	Field
6	M	678	678	65	65
	F	781	781	57	57
10	M	720	353	98	87
	F	694	655	86	108
14	M	732	347	144	100
	F	600	645	72	73
18	M	192	407	65	91
	F	365	563	28	69
22	M	145	458	108	81
	F	130	586	18	90
26	M	95	365	29	87
	F	62	418	32	80
30	M	28	415	14	79
	F	12	434	8	90
34	M	14	401	5	77
	F	2	458	1	82
38	M	2	398	0	69
	F	0	485	0	81
42	M	0	254	0	71
	F	0	345	0	42

Table 5.3 (contd.).

Weeks after salivary gland infection	Gender of tick	Total number of infected acini			
		Medium infections		Low infections	
		20°C	Field	20°C	Field
46	M	0	113	0	83
	F	0	214	0	46
50	M	0	105	0	85
	F	0	156	0	66
54	M	0	102	0	62
	F	0	93	0	50
58	M	0	85	0	68
	F	0	54	0	38
62	M	0	76	0	55
	F	0	49	0	45
66	M	0	39	0	43
	F	0	31	0	33
70	M	0	23	0	27
	F	0	20	0	13
74	M	0	9	0	19
	F	0	6	0	8
78	M	0	0	0	0
	F	0	0	0	0

5.4.4 Survival of *Theileria parva* in nymphal *Rhipicephalus appendiculatus* which had high and low infections

The survival of *T. parva* infection in the highly and lowly infected nymphae exposed to quasi-natural climatic conditions and a constant temperature of 20°C, 85% RH in the laboratory is shown in Figure 5.4 and Table 5.4.

High infections

The decrease in intensity of infection in the highly infected nymphal ticks kept under quasi-natural conditions was not as dramatic as in those kept under laboratory conditions at 20°C, 85% RH. However, falling intensity of infection was seen 6 weeks post-salivary gland infection. Fifty percent mortality level of *T. parva* in the highly infected nymphal ticks kept at 20°C, 85% RH was reached by 13 weeks after infection of the salivary glands and the infections died out by 26 weeks (Table 5.1). The duration of parasite survival in the salivary glands of the highly infected nymphal ticks was much longer under quasi-natural conditions than at 20°C, 85% RH. Fifty percent mortality of *T. parva* infection in the nymphal ticks kept under quasi-natural conditions was reached after 27 weeks post-salivary gland infection and the parasite could no longer be detected after 50 weeks (Table 5.1).

Low infections

Parasite survival in nymphal ticks having low infections followed a similar pattern to that seen in adult ticks having low infections (Figure 5.4). In nymphal ticks kept under quasi-natural conditions, intensity of infection remained remarkably constant until week 42 after salivary gland infection but the parasite died out by week 50. At

20°C, 85% RH a faster decline of infection was seen in the nymphal ticks and the infections died out by week 30 after salivary gland infection.

Again, there was a significant difference between the survival of the parasites kept in the field and those kept at 20°C, 85% RH in both cases ($p < 0.01$).

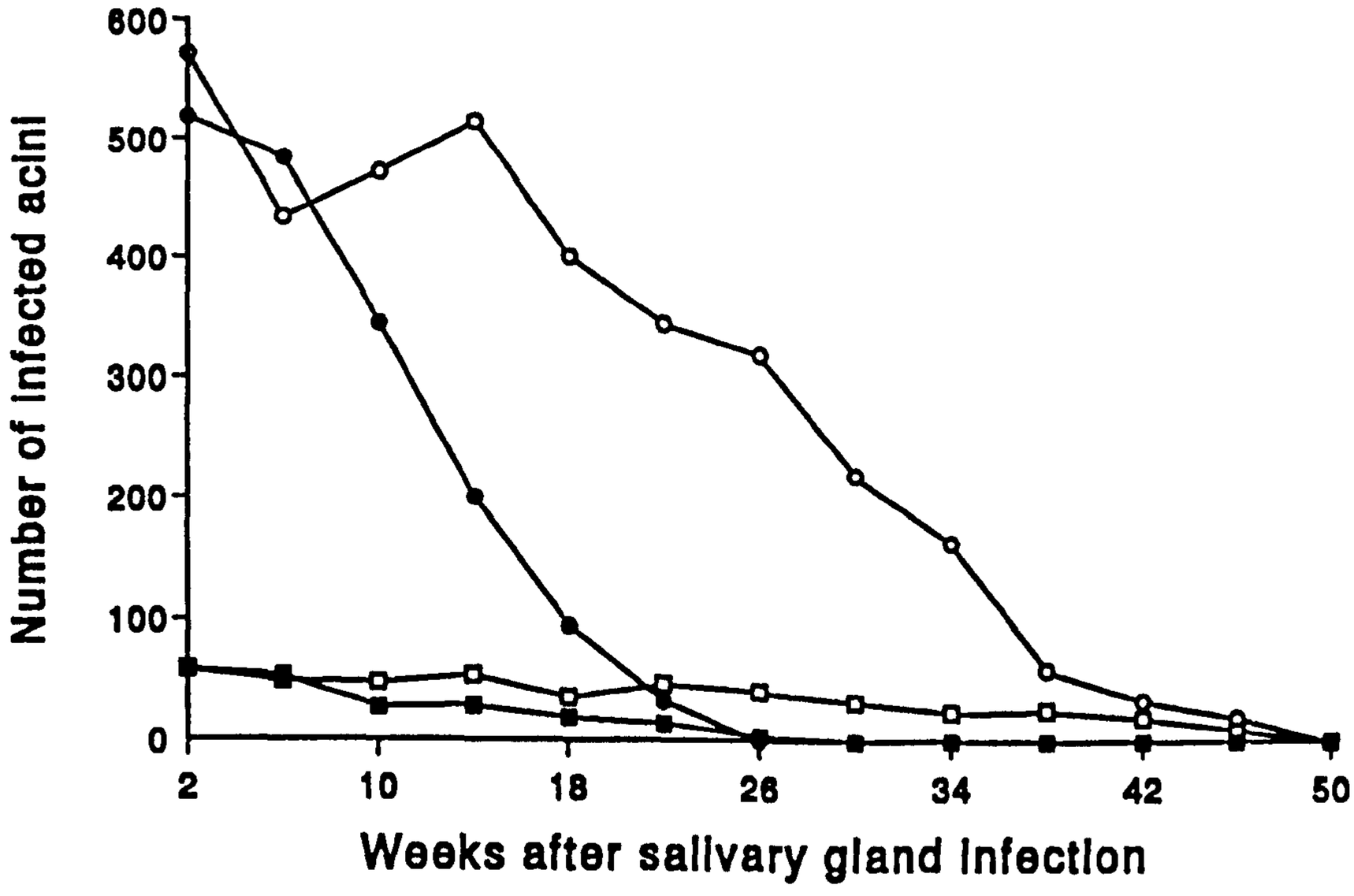


Figure 5.4. Survival of *Theileria parva* Muguga in the highly and lowly infected nymphal *Rhipicephalus appendiculatus* Muguga after exposure to different conditions.

Table 5.4. Survival of nymphal *Rhipicephalus appendiculatus* Muguga and *Theileria parva* Muguga parasite in their salivary glands after exposure to different conditions.

Weeks after infection of glands	Number of acini infected				Number of surviving nymphae			
	High infections		Low infections		Highly infected		Lowly infected	
	20°C	Field	20°C	Field	20°C	Field	20°C	Field
2	519	572	58	58	200	200	200	200
6	484	434	53	49	195	199	196	200
10	345	473	27	47	194	197	197	198
14	200	515	28	53	193	198	192	199
18	94	401	19	35	189	198	186	198
22	32	344	14	46	181	197	184	197
26	0	318	4	40	171	191	174	192
30	0	219	0	32	150	187	157	190
34	0	162	0	23	127	180	122	179
38	0	59	0	25	91	165	94	164
42	0	33	0	19	57	145	64	150
46	0	19	0	9	40	113	53	110
50	0	0	0	0	18	80	28	83
54	0	0	0	0	4	58	14	52
58	0	0	0	0	0	22	5	24
62	0	0	0	0	0	5	1	10
66	0	0	0	0	0	0	0	4
70	0	0	0	0	0	0	0	0

5.4.5 Survival of highly infected adult *Rhipicephalus appendiculatus* under laboratory and quasi-natural conditions

The results of the survival of highly infected *R. appendiculatus* adults under different climatic conditions is shown in Figure 5.5. No appreciable mortality occurred in ticks exposed to quasi-natural climatic conditions until 50 weeks after repletion of the nymphae when it increased rapidly. The most suitable laboratory conditions for survival of the ticks was the diurnal temperature rhythm of 13-23°C, 85% RH in which adult ticks survived for nearly as long as ticks kept under quasi-natural climatic conditions. Like in the ticks kept under quasi-natural climatic conditions, no appreciable mortality occurred in these ticks until 50 weeks after repletion of the nymphae. The third most favourable condition for survival of the highly infected adult ticks was at 20°C constant temperature, 85% RH where appreciable mortality developed after 46 weeks. At 24°C, 85% RH, mortality was accelerated appreciably compared to the three most suitable conditions and occurred over a longer period such that there was no prolonged period with appreciable mortality. A similar pattern was noted when ticks were exposed to 23-30°C diurnal temperature rhythm, 85% RH where the survival duration was the shortest. Results of the analysis to determine the period to 50% and 100% mortality are shown in Table 5.5. When the male and female ticks were considered separately it was found that a similar pattern of survival to that of the combined male and female tick survival occurred for each sex (Table 5.6).

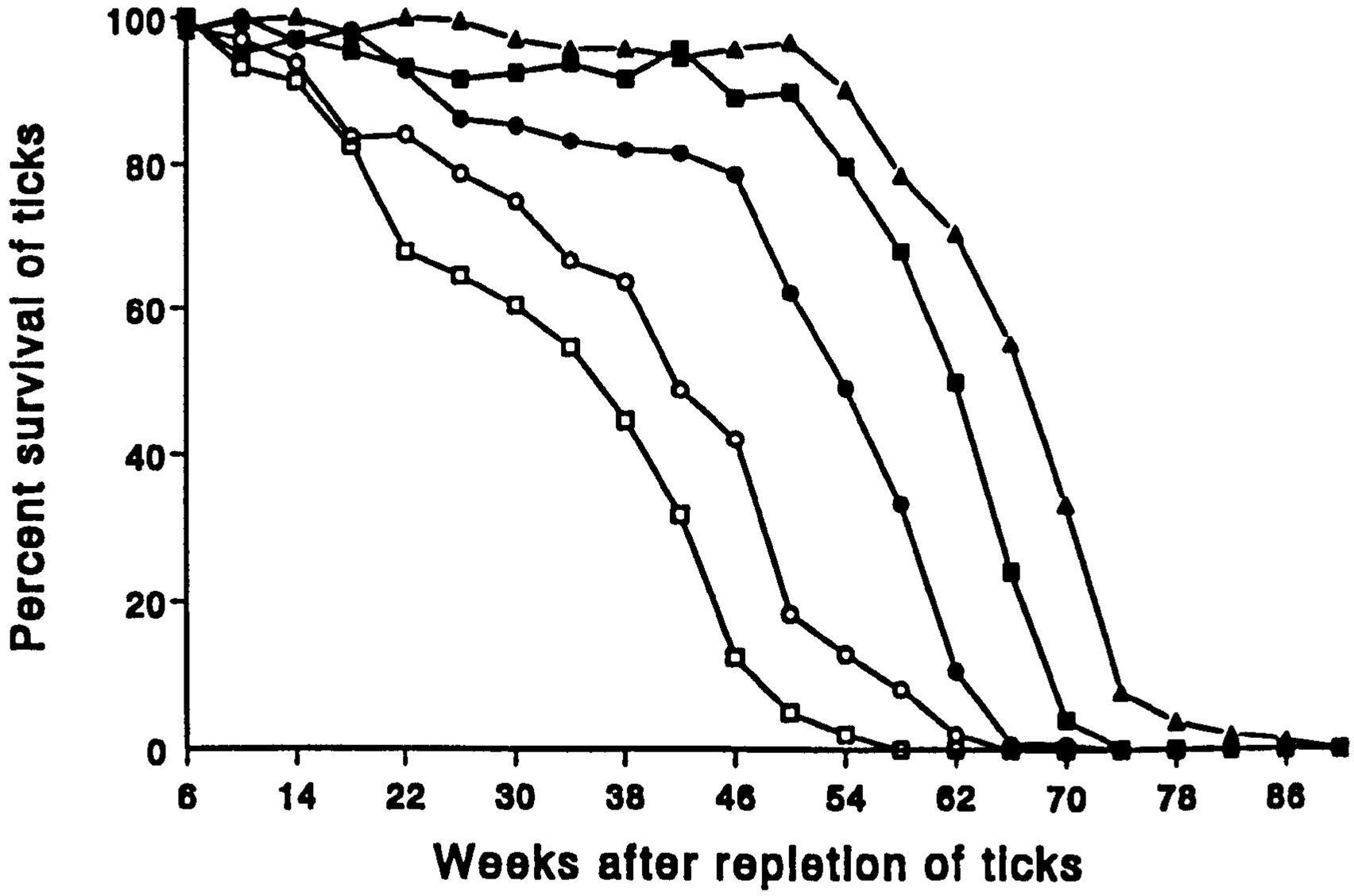


Figure 5.5. Survival of adult *Rhipicephalus appendiculatus* Muguga highly infected with *Theileria parva* Muguga after exposure to different laboratory and quasi-natural conditions.

Table 5.5. Period in weeks to fifty and hundred percent mortality of *Rhipicephalus appendiculatus* adults and nymphae highly infected with *Theileria parva* after exposure to different climatic conditions. All ticks kept in the laboratory were at 85% RH.

Instar	Conditions	Period to 50% mortality (weeks)	Period to 100% mortality (weeks)
Adults	Constant 20°C	54	68
	Constant 24°C	42	64
	Diurnal rhythm 13-23°C	62	72
	Diurnal rhythm 23-30°C	36	56
	Quasi-natural conditions	67	88
Nymphae	Constant 20°C	36	55
	Quasi-natural conditions	46	63

Table 5.6. Survival of adult *Rhipicephalus appendiculatus* Muguga highly infected with *Theileria parva* Muguga after exposure to different laboratory and quasi-natural conditions.

Tick gender	Conditions of exposure	Weeks after repletion of nymphae										
		6	10	14	18	22	26	30	34	38	42	46
Females	20°C	118	120	117	118	113	107	105	103	99	99	97
	24°C	120	116	113	95	104	96	92	79	78	61	50
	13-23°C	120	116	118	113	113	111	110	115	112	118	105
	23-30°C	120	110	106	98	79	74	72	66	53	39	16
	FIELD	118	120	120	119	120	120	117	117	116	113	115
Males	20°C	118	120	115	118	110	100	100	97	98	97	96
	24°C	117	117	112	106	98	93	88	81	75	56	51
	13-23°C	119	112	115	116	111	109	112	110	108	112	109
	23-30°C	120	114	113	100	84	81	73	65	54	37	14
	FIELD	118	119	120	116	120	119	116	113	114	114	115
Both	20°C	236	240	232	236	223	207	205	200	197	196	189
	24°C	237	233	225	201	202	189	180	160	153	117	101
	13-23°C	239	228	233	229	224	220	222	225	220	230	214
	23-30°C	240	224	219	198	163	155	145	131	107	76	30
	FIELD	236	239	240	235	240	239	233	230	230	227	230

Table 5.6 (contd.).

Tick gender	Conditions of exposure	Weeks after repletion of nymphae										
		50	54	58	62	66	70	74	78	82	86	90
Females	20°C	79	62	45	14	2	2	0	0	0	0	0
	24°C	24	16	9	4	0	0	0	0	0	0	0
	13-23°C	107	96	81	62	30	7	0	0	0	0	0
	23-30°C	8	4	0	0	0	0	0	0	0	0	0
	FIELD	118	110	95	84	66	44	11	7	4	3	0
Males	20°C	71	56	35	12	0	0	0	0	0	0	0
	24°C	20	15	11	1	0	0	0	0	0	0	0
	13-23°C	109	96	83	58	28	3	0	0	0	0	0
	23-30°C	4	1	0	0	0	0	0	0	0	0	0
	FIELD	114	107	94	86	67	36	8	2	1	0	0
Both	20°C	150	118	80	26	2	2	0	0	0	0	0
	24°C	44	31	20	5	0	0	0	0	0	0	0
	13-23°C	216	192	164	120	58	10	0	0	0	0	0
	23-30°C	12	5	0	0	0	0	0	0	0	0	0
	FIELD	232	217	189	170	133	80	19	9	5	3	0

5.4.6 Survival of adult *Rhipicephalus appendiculatus* having medium and low infections under laboratory and quasi-natural conditions

The survival of adult *Rhipicephalus appendiculatus* having medium and low infections under various laboratory and quasi-natural climatic conditions is shown in Figure 5.6. Table 5.7 contains the results for males and females separately. The time to 50% mortality in ticks kept at 20°C, 85% RH having medium and low infections was 52 weeks in both cases. The time in weeks to 50% mortality in the same ticks but kept in the field was 64.5 and 68.5 respectively. The time to 100% mortality in ticks kept at 20°C, 85% RH having medium and low infections was between 66 and 70 weeks in both cases. The duration of survival of ticks kept in the field was much longer than that of ticks kept at 20°C, 85% RH in both categories of ticks. The time to 100% mortality of both categories was 102 weeks after feeding to repletion for both male and female ticks.

The pattern of survival in the adult ticks having medium and low infections kept under the two conditions (20°C, 85% RH and field) was very similar to the pattern of survival in adult ticks having high infections kept under the same conditions (Figure 5.5) even though the former category of ticks survived for a marginally longer time.

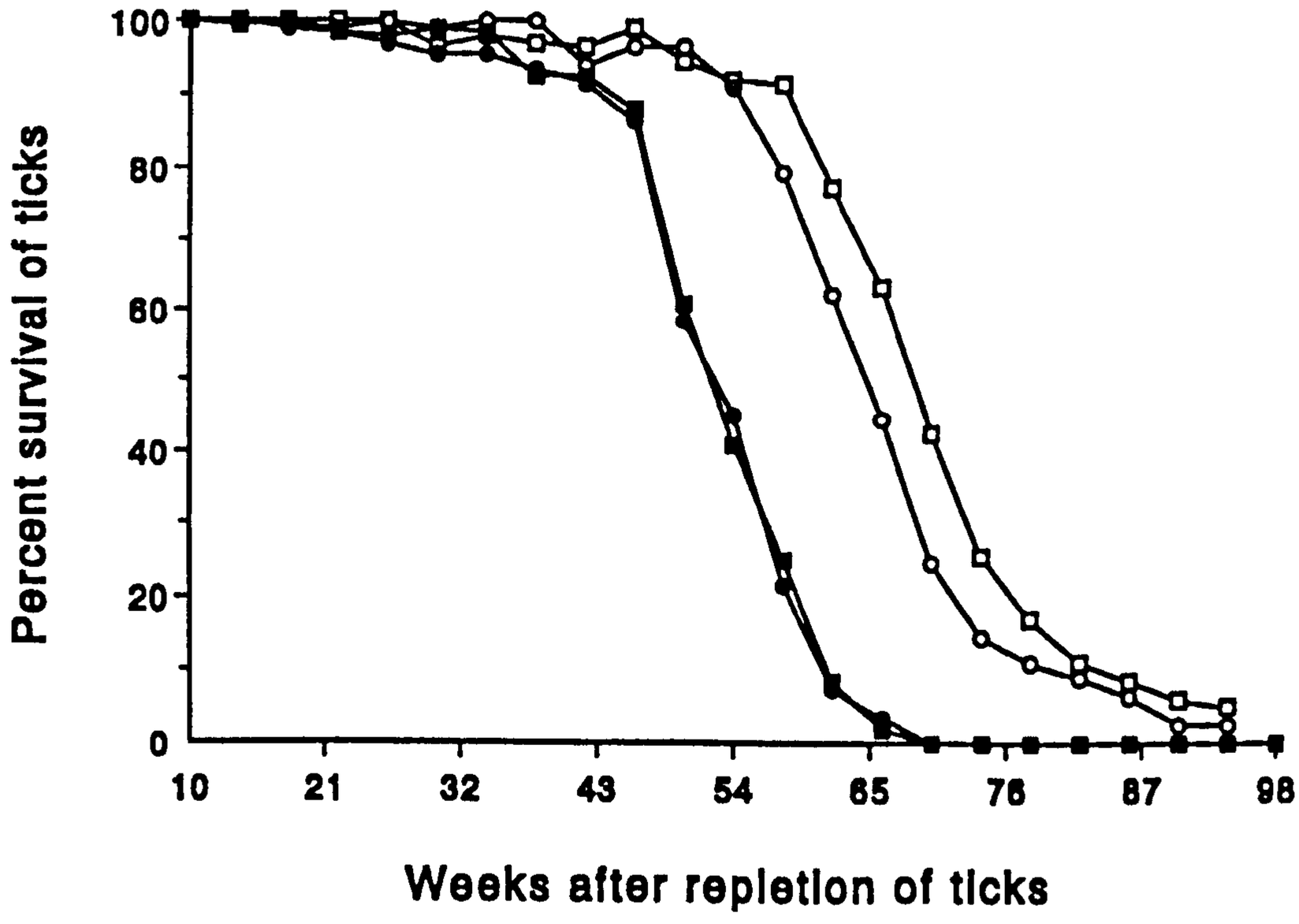


Figure 5.6. Survival of adult *Rhipicephalus appendiculatus* Muguga having medium and low infections of *Theileria parva* Muguga after exposure to different climatic conditions.

Table 5.7. Survival of adult male and female *Rhipicephalus appendiculatus* Muguga having medium and low infections of *Theileria parva* Muguga after exposure to different climatic conditions. M = adult male ticks; F = adult female ticks.

Weeks after repletion of ticks	Gender of tick	Number of surviving adult ticks			
		Medium infecteds		Lowly infecteds	
		20°C	Field	20°C	Field
10	M	100	100	100	100
	F	100	100	100	100
14	M	100	100	99	100
	F	100	100	100	100
18	M	98	99	100	100
	F	100	100	100	100
22	M	98	100	98	100
	F	99	98	99	100
26	M	96	100	98	100
	F	98	100	98	100
30	M	94	99	98	97
	F	97	99	100	96
34	M	95	100	100	99
	F	96	100	97	97
38	M	91	100	94	97
	F	96	100	91	97
42	M	90	94	93	99
	F	93	94	92	94
46	M	84	97	82	98
	F	89	96	94	100

Table 5.7 (contd.).

Weeks after repletion of ticks	Gender of tick	Number of surviving adult ticks			
		Medium infecteds		Lowly infecteds	
		20°C	Field	20°C	Field
50	M	57	95	61	92
	F	60	98	61	97
54	M	42	87	39	92
	F	48	95	43	92
58	M	20	80	22	92
	F	23	79	28	91
62	M	6	59	7	66
	F	9	66	10	89
66	M	3	42	1	65
	F	4	47	3	62
70	M	0	27	0	40
	F	0	22	0	45
74	M	0	14	0	19
	F	0	15	0	32
78	M	0	12	0	12
	F	0	10	0	22
82	M	0	10	0	9
	F	0	8	0	13
86	M	0	7	0	7
	F	0	6	0	10
90	M	0	3	0	5
	F	0	2	0	7

Table 5.7 (contd.).

Weeks after repletion of ticks	Gender of tick	Number of surviving adult ticks			
		Medium infecteds		Lowly infecteds	
		20°C	Field	20°C	Field
94	M	0	3	0	4
	F	0	2	0	6
98	M	0	2	0	2
	F	0	2	0	5
102	M	0	1	0	1
	F	0	2	0	4
106	M	0	0	0	0
	F	0	0	0	0

5.4.7 Survival of highly and lowly infected nymphal *Rhipicephalus appendiculatus* under laboratory and quasi-natural conditions

The results of the survival of nymphal *R. appendiculatus* having high and low infections is shown in Table 5.4 and Figure 5.7. Nymphal ticks kept under quasi-natural conditions showed a longer survival than ticks kept under constant temperature of 20°C, 85% RH in the laboratory. The periods for 50% and 100% mortality of the highly infected nymphal ticks kept under different conditions is shown in Table 5.5.

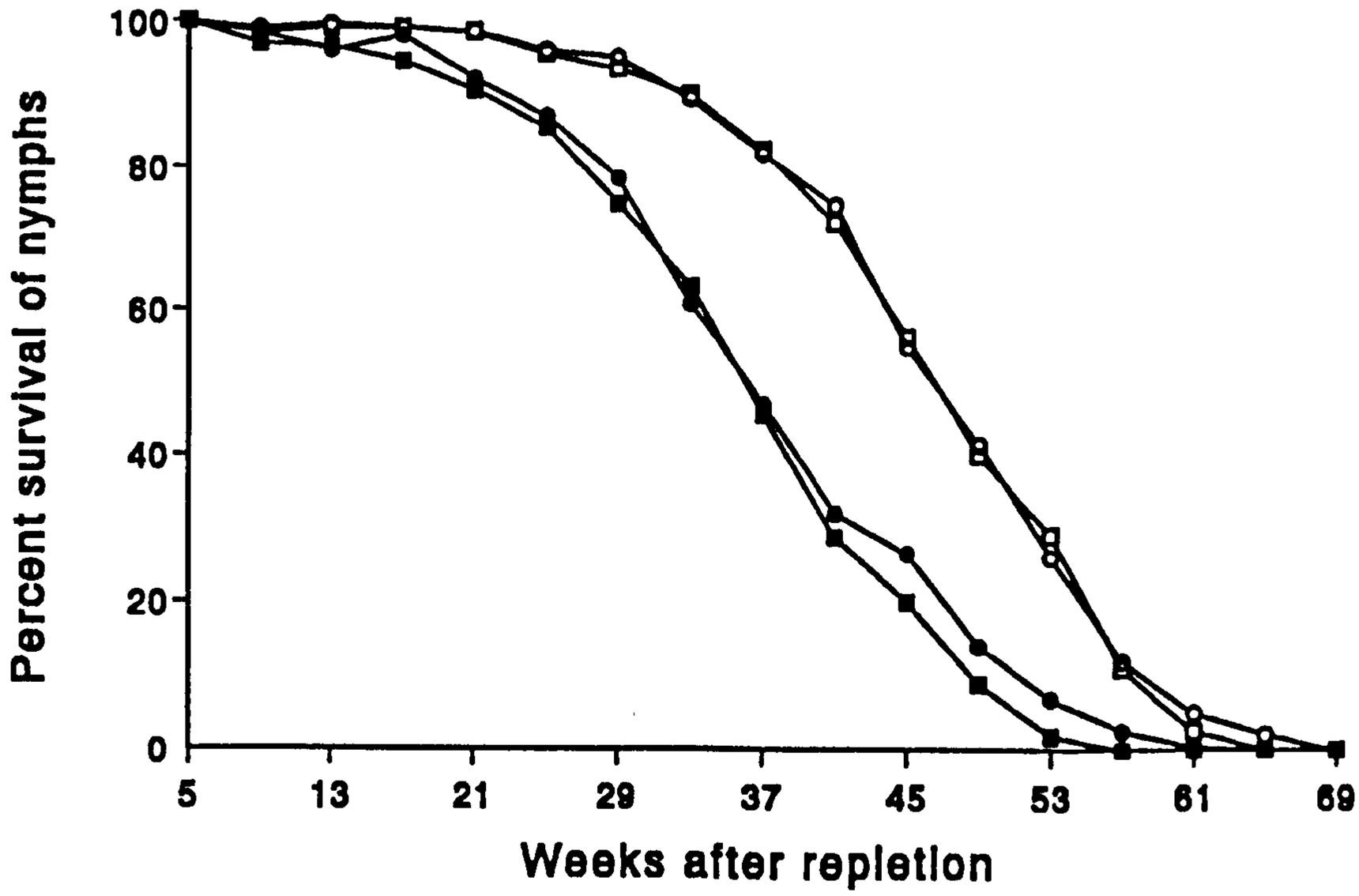


Figure 5.7. Survival of the highly and lowly infected nymphal *Rhipicephalus appendiculatus* Muguga.

5.5 DISCUSSION

Although there have been several studies on the survival of *R. appendiculatus* and *T. parva* within the tick salivary glands under various climatic conditions, this is the first to consider the influence of infection levels on the survival of the parasite and the tick. In addition, it is the first to not only compare survival between infected nymphal and adult ticks, but also under laboratory and field conditions.

The survival of ticks under climatic conditions reproduced in the laboratory was shorter than that under field conditions; however, adult ticks exposed to the most favourable laboratory condition of diurnal temperature rhythms of 13-23°C and 85% RH humidity showed a comparable survival pattern to ticks exposed to field conditions. The survival of the parasites in the ticks kept in the laboratory was much shorter than under field conditions. This phenomenon was most marked in adult ticks with high infections, where infection collapsed under all laboratory conditions between 10 and 18 weeks after the salivary glands became infected. Such a collapse in infection levels was not seen in the field ticks until much later. A question arises as to how survival of the ticks can be similar in the laboratory and the field but the survival of the parasite is much better under field conditions. Further investigations have to be undertaken to determine the factors which allow better survival of *T. parva* in the salivary glands of ticks exposed to field conditions than in laboratory-maintained ticks.

Another factor noted in field exposed ticks but not in the laboratory exposed ticks was an infection-density influence on the survival time of parasites. While there was great reduction in infection intensity of *T. parva* in ticks exposed to field conditions

which had high and medium infection intensities over the exposure period, there was little or no decrease in the intensity of infection in ticks with low infection. Hence the conditions in ticks with high and medium infections are not as favourable for parasite survival as in ticks with low infections. As ticks with low infection intensities are normal in the field, this would appear to favour parasite survival. This may be due to the overdispersed nature of *T. parva* infections. Further analysis is required to consider how overdispersed distributions change subject to mortality.

When the survival of adult female and male *R. appendiculatus* were compared a similar pattern of survival was seen. Also the survival of *T. parva* parasites within salivary glands of male and female ticks was compared and a similar pattern was found. As expected, the nymphal ticks showed a shorter survival period than either male or female ticks and *T. parva* infection survived longer in adults than nymphae exposed to field conditions. However, under laboratory conditions, the infections in nymphs survived as long or longer than infection in highly infected adults. This was due to the infection-density dependent survival already discussed since highly infected nymphae have much lower intensity of infections than highly infected adult ticks. In numerical terms, under quasi-natural conditions, *T. parva* infections survived in highly infected adult ticks for 78 weeks post-salivary gland infection while the ticks survived for 88 weeks after feeding to repletion as nymphae. In the case of highly infected nymphal ticks *T. parva* infection survived for 50 weeks after infection of the salivary glands and the ticks themselves for 63 weeks after repletion of the larvae. The duration of survival of *T. parva* in adult ticks was similar to that obtained by Newson *et al.* (1984) and Young *et al.* (1983b) for a slightly higher altitude at Muguga, Kenya where 19 months was the survival period. The results

obtained here that the *T. parva* parasite will survive for about one year in nymphal ticks is new data.

From the tick survival studies of Branagan (1973), Punyua (1985), Newson *et al.* (1984) and in this study, it should be possible to model the survival of different instars in East Africa. Although similar studies have been carried out in central and southern Africa (Short, Floyd, Norval & Sutherst, 1989; Pegram & Banda, 1990; Berkvens, 1990), the situation is complicated there by the occurrence of behavioural diapause in adult ticks. In addition, this study and those of Young *et al.* (1983b), Newson *et al.* (1984), and Young *et al.* (1987) should allow accurate modelling of the transmission of *T. parva* in eastern Africa. For example, Medley *et al.* (1993) have constructed preliminary transmission models which could be further developed using this information. For central and southern Africa where diapause occurs in *R. appendiculatus* population, the effect of diapause on the survival of the adult tick and its *T. parva* infection would have to be investigated (Young *et al.*, in preparation b).

There has always been some debate over survival of *T. parva* in areas of seasonal activity of the tick vector, whether the cattle host, in the form of carrier animals, or the tick harboured infection during non transmission. This work demonstrates that it is quite possible for the tick (adult and nymphal) to act as reservoirs.

CHAPTER 6

MODELLING *THEILERIA PARVA* INFECTIONS IN *RHIPICEPHALUS APPENDICULATUS*

6.1 INTRODUCTION

In this chapter, the effects of various parameters investigated in the experimental phase of this study, described in Chapters 2 to 5, are analysed statistically.

The population dynamics of *T. parva* in the mammalian and tick hosts depend on several factors. These factors influence the dynamics of the parasite at the individual or group host level. Factors affecting infection in mammalian hosts as a group include tick counts on cattle, tick infection rates, success of transmission, antibody prevalence, case fatality rates and disease incidence. Infection patterns in the mammalian host population can be assessed from parameters such as antibody prevalence, case fatality rates, disease incidence, immunity to homologous strains and host resistance to ticks. Within individual hosts, patterns of infection can be assessed from parameters such as immunity to infection and parasite densities. Infection dynamics in mammalian hosts is discussed in detail in Norval *et al.*(1992a). The dynamics of *Theileria* infections in ticks is influenced by factors such as tick counts on cattle, tick infection rates and the success of transmission to ticks. This study investigated only some of the poorly understood processes controlling the degree of *T. parva* infections and the subsequent transmission success by the rhipicephalid tick vectors. Statistical models of the parasite in the tick vector were developed from the data obtained using the logistic and Poisson regression analyses and the relative importance of some factors in determining the infection levels developing in the vectors evaluated.

6.2 EXPERIMENTAL DESIGN AND ANALYSIS

6.2.1 Tick infection

Infection of the various rhipicephalid tick instars and stocks with the Muguga and Boleni stock of *T. parva* is described in Section 2.4. Sixty nymphal or 30 male and 30 female ticks, randomly picked from respective batches, were assessed for prevalence and abundance of infection after Feulgen's staining (Section 2.5).

6.2.2 Data analysis

The effect of the different categorical variables on the fit of the respective models was assessed using the computer software SAS/STAT® Release 6.03 Edition (SAS Institute Inc., SAS Campus Drive, Cary NC 27513).

The logistic regression analysis was used to model the effect of the various variables on the prevalence of infection or proportion of surviving ticks while the intensity of infection, being measured by simple count data, was modelled by the Poisson regression analysis. Each individual variable or group of dummy variables were added sequentially to the null model and their improvement to the model fit assessed, based on the change in the residual scaled deviance compared to that of the residual degrees of freedom.

Logistic regression analysis

Prevalence data (expressed as proportions) vary between 0 and 1. Logistic regression is basically linear regression performed on logit transformed data, where

the error distribution is assumed known and binomial. The logit transformation is given as:

$$\text{logit}(P) = \log \frac{P}{1-P} \qquad (1)$$

where P is the probability of a tick being infected (= proportion of ticks infected in a batch).

An analysis of tick instars/gender and time post-infection can be defined as:

$$\text{logit}(P_{ij}) = \alpha_i + \beta x_j \qquad (2)$$

where P_{ij} is the proportion of ticks infected of instar/gender i on day j post-infection, α_i is a parameter to be estimated and is the intercept associated with, for example, tick instar and β is the slope to be estimated determining, for example, the effect of days post-infection. The expected values can be calculated as:

$$P = \frac{e^{\alpha_i + \beta x_j}}{1 + e^{\alpha_i + \beta x_j}} \qquad (3)$$

The variables were then included into the model and the model tested for better fit using the -2 log likelihood method. For a full account of logistic regression see Freeman (1987).

The OV1, OV2 and OV3 macro programmes within GLIM were used to account for overdispersion of responses under the binomial distributional assumptions. The OV1, macro is for the constant overdispersion parameter (residual deviance over residual degrees). The OV2 and OV3 macros are overdispersion parameters (model II and III respectively), both due to Williams (1982). OV2 is a beta-binomial analogue and OV3 is a logistic-normal analogue.

Poisson regression analysis

As a good first approach to modelling count data and means of counts, the Poisson distributional assumptions were made in modelling the intensity of *T. parva* infection in the various *R. appendiculatus* tick batches. The outcome (dependent) variable was defined as number of infected acini. A log-linear model structure was used, that is,

$$\log \mu = x\beta \qquad (4)$$

where μ is the expected mean of the dependent variable (number of infected acini), x are the values of the explanatory variables and β the parameters to be estimated. Poisson distribution is dealt with in more detail by Agresti (1990). The various variables were analysed separately for their effect on the fit of the respective models using the GLIM software.

Individual variables (or groups of dummy variables) were fitted sequentially to the minimal (null) model having the intercept only and their effect on the model assessed. The significance of additional variables towards the improved fit of the overall model was assessed by means of the change in scaled deviance between the

larger and smaller nested models. Under maximum likelihood methods and the distributional assumptions made concerning the data, the difference in scaled deviance follows a chi-square distribution with degrees of freedom equal to the difference in numbers of variables between the models.

The following two methods were applied in evaluating the distributional assumptions.

i) A Q-plot of the standardized residuals using a macro software (B. J. Francis, Centre for Applied Statistics, University of Lancaster, UK) within GLIM was drawn. If the distributional assumptions are correct, the standardized residuals follow a Normal distribution and the Q-plot produces a straight line of slope 1. Generally, coefficient values less than 0.9500 were considered poor fits.

ii) Plotting the standardized (Pearson's) residuals against the fitted values of the model and those of the independent variables. The factors of concern in assessing standardized residuals were their actual magnitude and distribution over the fitted values and their trend over the independent variables. Generally, values of less than -3 or greater than +3 were considered to be outliers.

The OV4 and OV5 macro programmes within GLIM used to account for overdispersion of responses under the Poisson distributional assumptions. The OV4 method uses a fixed overdispersion parameter to deflate the residual scaled deviance by inflating the parameter variance estimates. This was essentially the same as making the assumption that all of the unexplained deviance was due to overdispersion, a relatively conservative approach since there is seldom a perfectly

fitting model. The OV4 method was thus only used to evaluate the significance of the parameters to the fit of the models. The OV5 assumes that the distribution of errors is that of the negative binomial distribution. Finally, the acini counts in each data file were transformed into the log scale to fit the Poisson distribution and the model fitting procedures described above repeated.

6.2.3 Data

Comparative nymphal and adult tick transmission

There were a total of 2,400 individual tick observations originating from 5 batches from each of the 4 steers. The results of the observations are summarised in Table 3.2. This data set was also used in plotting and assessing the distributions of infection intensities.

Each tick assessed within a batch of ticks represented an independent observation within that batch, however the observations were expected to be correlated as each batch came from the same bovine host on the same day, hence batch was considered as a potentially confounding variable. Since there was no particular relationship or trend implied between prevalence of infection and batch, the initial parameterization of batch created 4 (=n-1) "dummy variables" in tick-stage models. Given that the 5 batches dropped replete from each steer on days 16 through 20 post-infection, a continuous variable of "days post infection" was also considered. The bovine host was also treated as a confounding variable in assessing the effect of tick stage on prevalence and abundance of infection. Since the bovine host is a classification variable its effect was accounted for by inclusion of 3 (=n-1) dummy variables in models for tick stage.

Bovine host variable was offered as a categorical variable with four levels (three dummy variables were used and the fourth represented by the intercept). Likewise for instar, the parameter estimates for each factor (level) compared was males to nymphs and females to nymphs. Differences in residual scaled deviance between models containing all three instars separately, and that containing either males and females together or nymphs and males together were assessed for differences in abundance of infection between the instars and gender. The remaining variables were assessed likewise for their effect on the fit of the model.

Manipulation of the original data file to create three separate files was necessitated by the death of one experimental animal on the last day of collection of replete ticks. Thus, even though the animal was lost, replete ticks were collected on that day and their infection levels assessed. The SAS/STAT® Release 6.03 Edition programme was used to create three data files from the original data file. For convenience these files were referred to as Datafile1 (containing all recorded piroplasm values, that is, 2,400 observations), Datafile2 (in which observations with missing piroplasm values were deleted, hence had 2,280 observations) and Datafile3 (in which the missing piroplasm value was imputed hence had 2,400 observations). Each of these files were analysed separately for model fit.

Vector competence

There were a total of 19 tick batches for each stock infected with *T. parva* Muguga. Three were first generation ticks originating from 3 bovine hosts and 16 were second generation ticks originating from 4 hosts. Four tick batches feeding to repletion on

two animals infected with *T. parva* Boleni were analysed. Results of the observations are summarised in Table 4.1.

The independent variables analysed were tick stock, parasite stock, tick instar/gender, bovine host effects, batch of origin and day of repletion after infection of the bovine host.

Parasite and infected tick vector survival

The survival of *T. parva* and infected adult and nymphal ticks was monitored at monthly intervals over periods of up to two years (Chapter 5). There were three categories of infection in adult tick batches and two in the nymphal ticks (Section 5.3). Summarized results of observations are given in Tables 5.2, 5.3, 5.4, 5.6 and 5.7. The independent variables assessed were: instars, levels of infection, conditions of exposure and time over tick exposure to the various conditions .

6.3 RESULTS

6.3.1 Comparison of nymphal and adult tick transmission of *T. parva* infections

Distributions of T. parva *infection intensities*

A typical example of the distributions of *Theileria parva* infection intensities in *Rhipicephalus appendiculatus* was obtained from ticks that fed to repletion on the steer numbered BK97 on day 16 post-infection. The frequency distribution plot is shown in Figure 6.1. The plot exhibited a typically overdispersed parasite population distribution pattern for all the three instars with majority of ticks having very little or no infection but a minority having very high infections.

When all the ticks that fed to repletion over 5 consecutive days on four cattle were considered, the basic pattern of the frequency plot remained the same (Figure 6.2). Thus about 83%, 60% and 45% of female, male and nymphal ticks respectively each had 10 or less infected acini in their salivary glands. Even though the distribution patterns were similar, each tick instar and sex exhibited its own unique characteristics.

In order to assess the effect of the differing number of type III acini on the intensity of infection developing in the different instars, it was appropriate to consider the proportion of acini infected based on the average number of type III acini available for infection (Table 3.5). The result of the plot of the proportion of ticks infected compared to possible acini infected is given in Figure 6.3. Under this consideration, about 80% of nymphal , 85% of female and 93% of male ticks would have 10% or less of their type III acini infected. The instars also exhibited differences in the

distribution patterns of ticks falling in the intermediate region of infection levels. Nymphal ticks appeared to have higher infected individuals in this category compared to both adult ticks. Using the average number of type III acini (Table 3.5), and the total number of infected acini during the experiment, the probability of an acinus being infected for nymphae males and females were 0.064, 0.023 and 0.037 respectively.

Prevalence of infection

Effect of instar/gender: the results of the log likelihood values when different parameters were used in the models are given in Table 6.1. The instar/gender variable was included into the model first to develop the basic model. Incorporating a potential difference over tick instars significantly improved the fit of the model ($p < 0.001$). In order to determine whether there were significant differences between the male and female instars, a variable "adult" was created and modelled alone. The resultant model was a significant improvement ($p < 0.01$). This meant that the difference between prevalence of infection in the male and female instars was statistically significant. In a similar manner, a statistically significant difference ($p < 0.001$) between prevalence of infection in the male and nymphal instars was also demonstrated, implying that the difference in infection levels between female and nymphal ticks was also significant ($p < 0.001$).

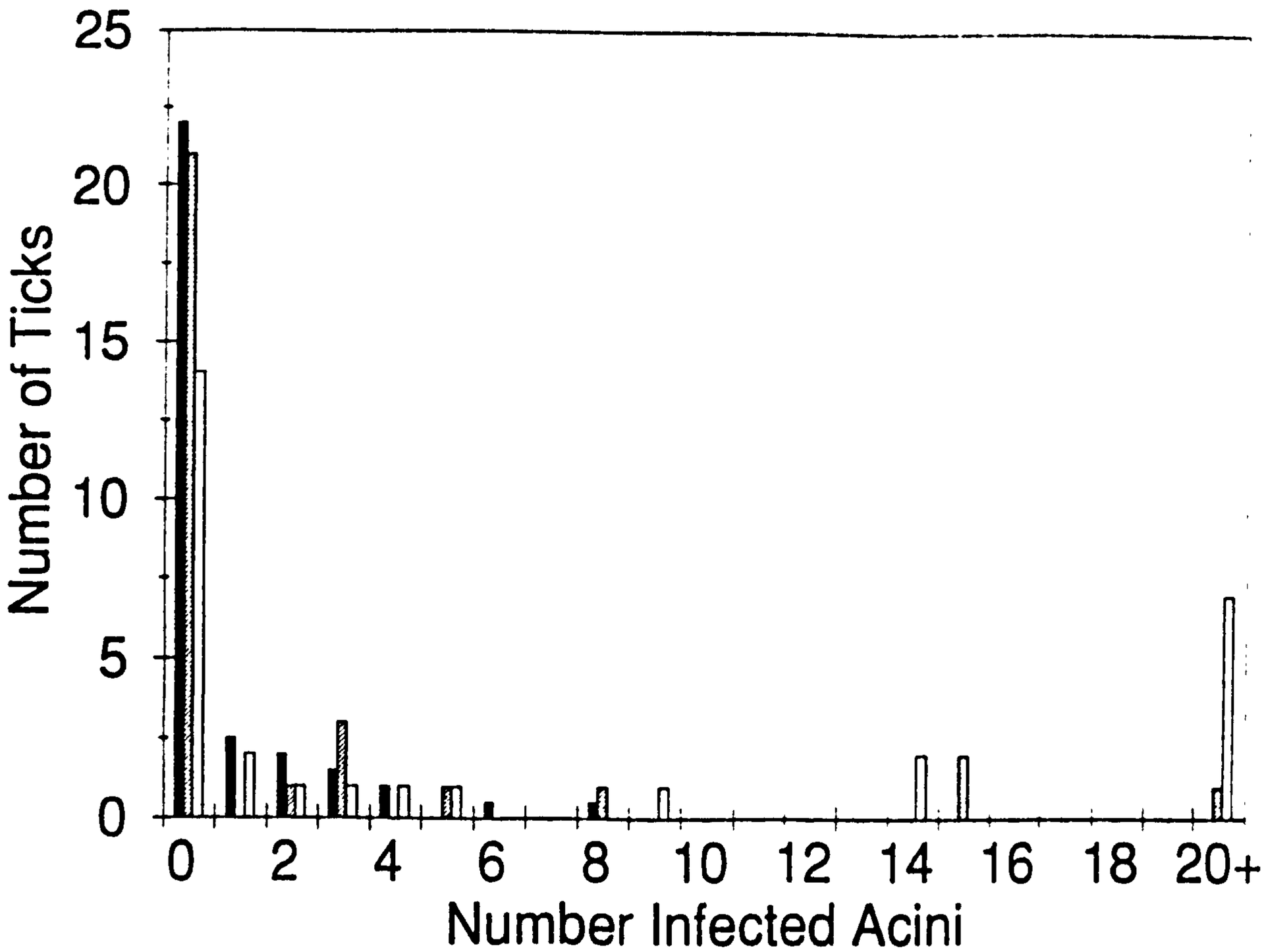


Figure 6.1. Frequency distribution plot of *Theileria parva* Muguga infection intensities in *Rhipicephalus appendiculatus* nymphae (▭) and adult males (▨) and females (■). The ticks fed to repletion as larvae or nymphae on a bovine host suffering acute infection on day 16 after sporozoite stabilate inoculation.

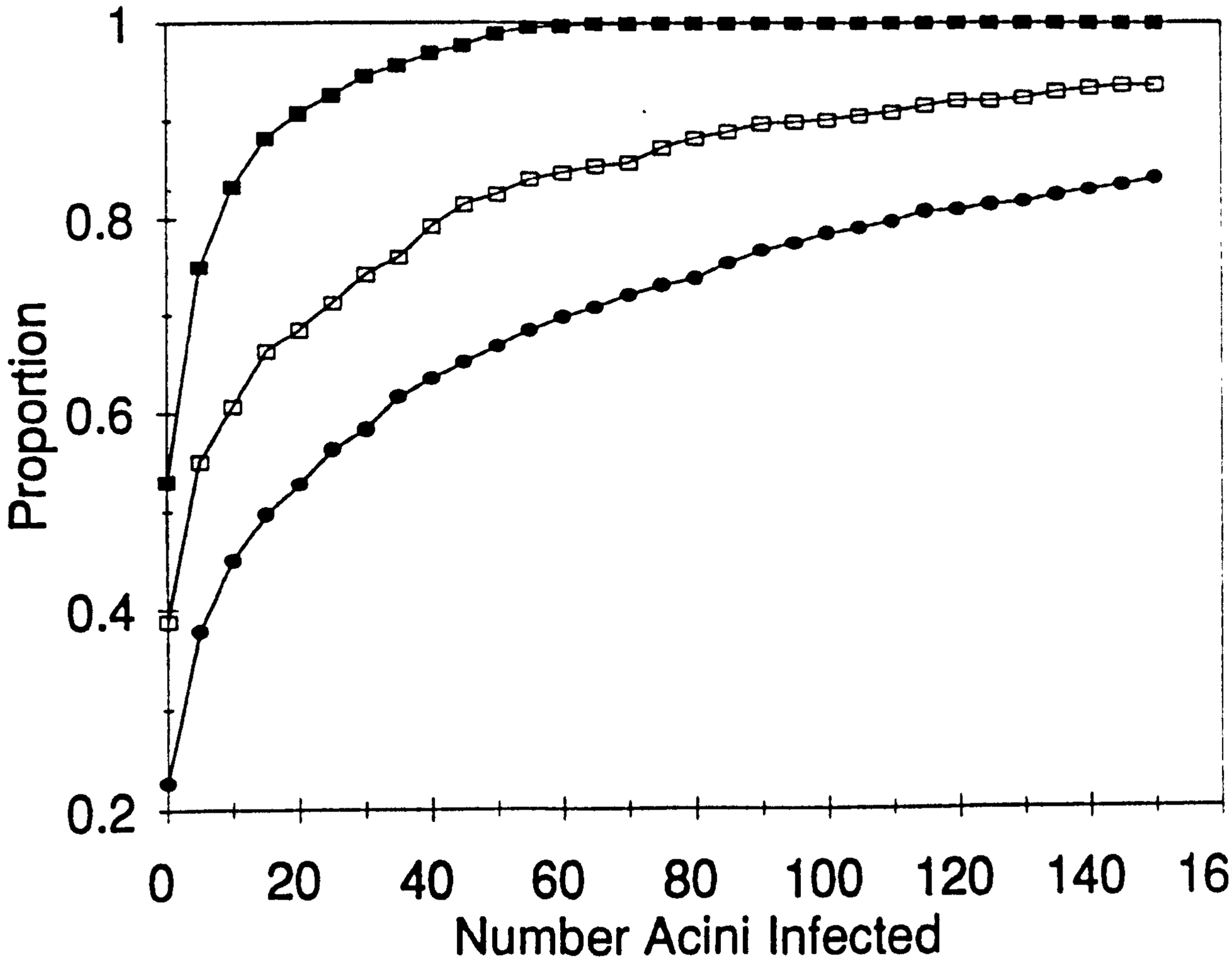


Figure 6.2. Frequency distribution plot of *Theileria parva* Muguga infection intensities in *Rhipicephalus appendiculatus* nymphae (●) and adult males (□) and females (■) plotted as a proportion of infected ticks against the number of infected acini. Data are from five tick batches that fed to repletion as larvae or nymphae from each of four cattle on days 16 to 20 after sporozoite stabilate inoculation.

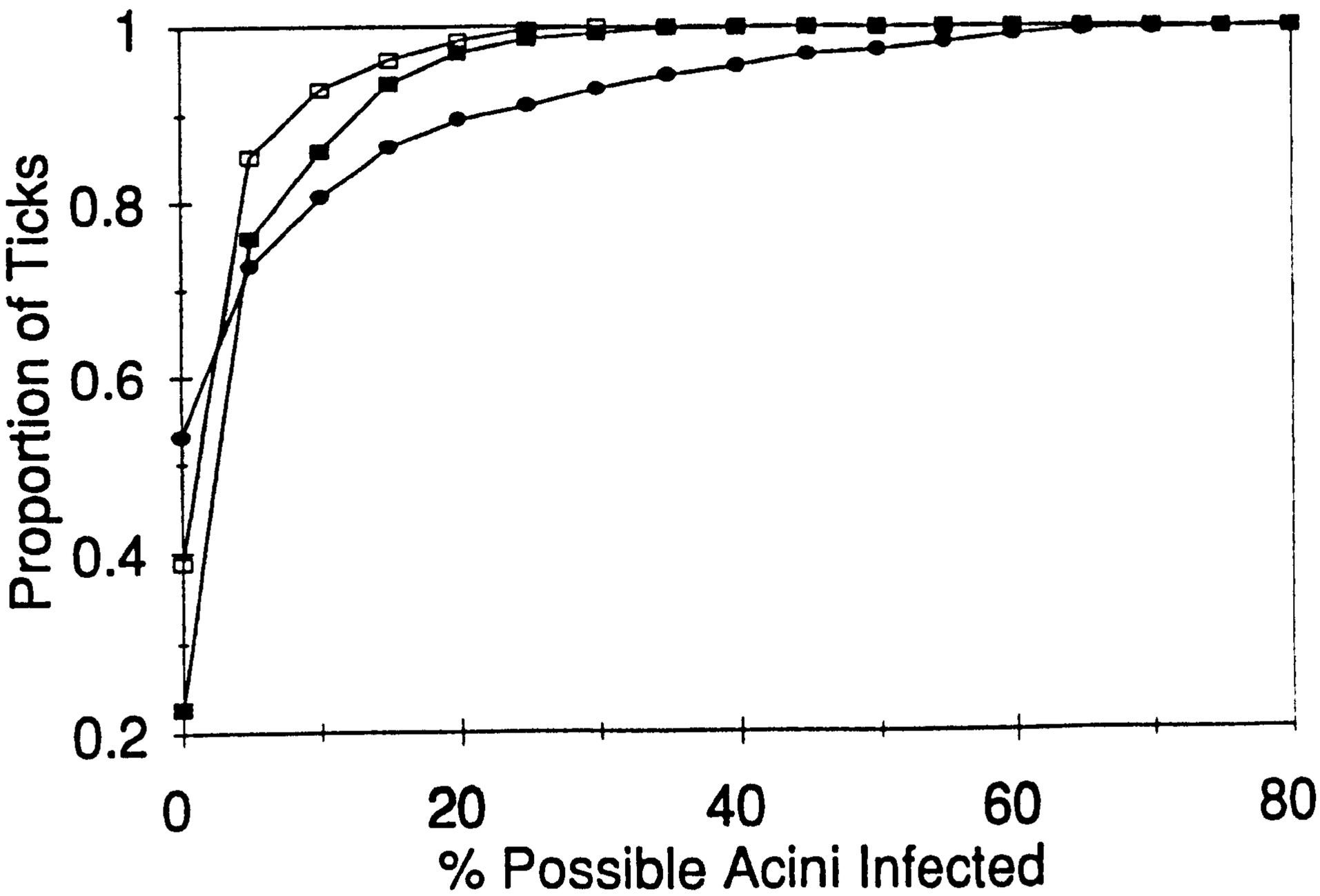


Figure 6.3. Frequency distribution plot of *Theileria parva* Muguga infection intensities in *Rhipicephalus appendiculatus* nymphae (●) and adult males (□) and females (■) plotted as a proportion of infected ticks against the proportion of acini available for infection. Data are from five tick batches that fed to repletion as larvae or nymphae from each of four cattle on days 16 to 20 after sporozoite stabilate inoculation.

Table 6.1 Log likelihood values resulting from modelling different variables/parameters. B - batch; BP1 to 5 - reparameterized batch variable combination, respectively B2+B3, B3+B4, B4+B5, B3+B5, and B3+B4+B5; C - bovine; Piro, Piro2 - respectively, linear and quadratic terms of piroplasm density; DayPI, Day2, Day3 - days post infection as linear, quadratic and cubic terms respectively.

Model parameters	-2 Log L	D.F.
Male, Female	162.426	2
Female	128.514	1
Adult	125.989	1
Male, Female, B2, B3, B4, B5	399.432	6
Male, Female, B3, B4, B5	359.440	5
Male, Female, BP1, B4, B5	366.929	5
Male, Female, B2, BP2, B5	397.292	5
Male, Female, B2, B3, BP3	393.735	5
Male, Female, B2, BP4, B4	398.576	5
Male, Female, B2, BP5	393.651	4
Male, Female, C2, C3, C4	167.245	5
Male, Female, Piro	210.604	3
Male, Female, Piro, Piro2	271.438	4
Male, Female, Piro, Piro2, C2, C3, C4	364.323	7
Male, Female, B2, B3, B4, B5, C2, C3, C4	404.775	9
Male, Female, B2, BP5, C2, C3, C4	398.981	7
Male, Female, B2, B3, B4, B5, C2, C3, C4, Piro, Piro2	392.576	11
Male, Female, B2, BP5, C2, C3, C4, Piro, Piro2,	390.087	9
Male, Female, DayPI	333.260	3
Male, Female, DayPI, Day2	396.776	4
Male, Female, DayPI, Day2, Day3	399.081	5
Male, Female, DayPI, Day2, C2, C3, C4	402.113	7

Effect of batch: addition of the four batch dummy variables improved the fit of the basic model significantly ($p < 0.01$), however, this was without any indication of where the differences between the batches existed. The results of sequential combinations of pairs of adjacent batches are given in Table 6.2. The two differences which were not significant for individual comparisons were between batches 3 and 4, and 3 and 5. The combination of batch 3 and 5 was also tested because the difference between batch 4 and 5 was marginally significant. Since batch was a surrogate representation of increasing days post-infection, batches 3, 4 and 5 were combined and assessed to test if this combination increased the efficiency of the model. The combination B3 + B4 + B5 (versus B1, B2) resulted in a marginally less significant difference ($p < 0.05$) implying that it would prove more efficient in the modelling process to combine the 3 dummy variables in a reparameterization, that is, B1+B2 versus B3, B4, B5.

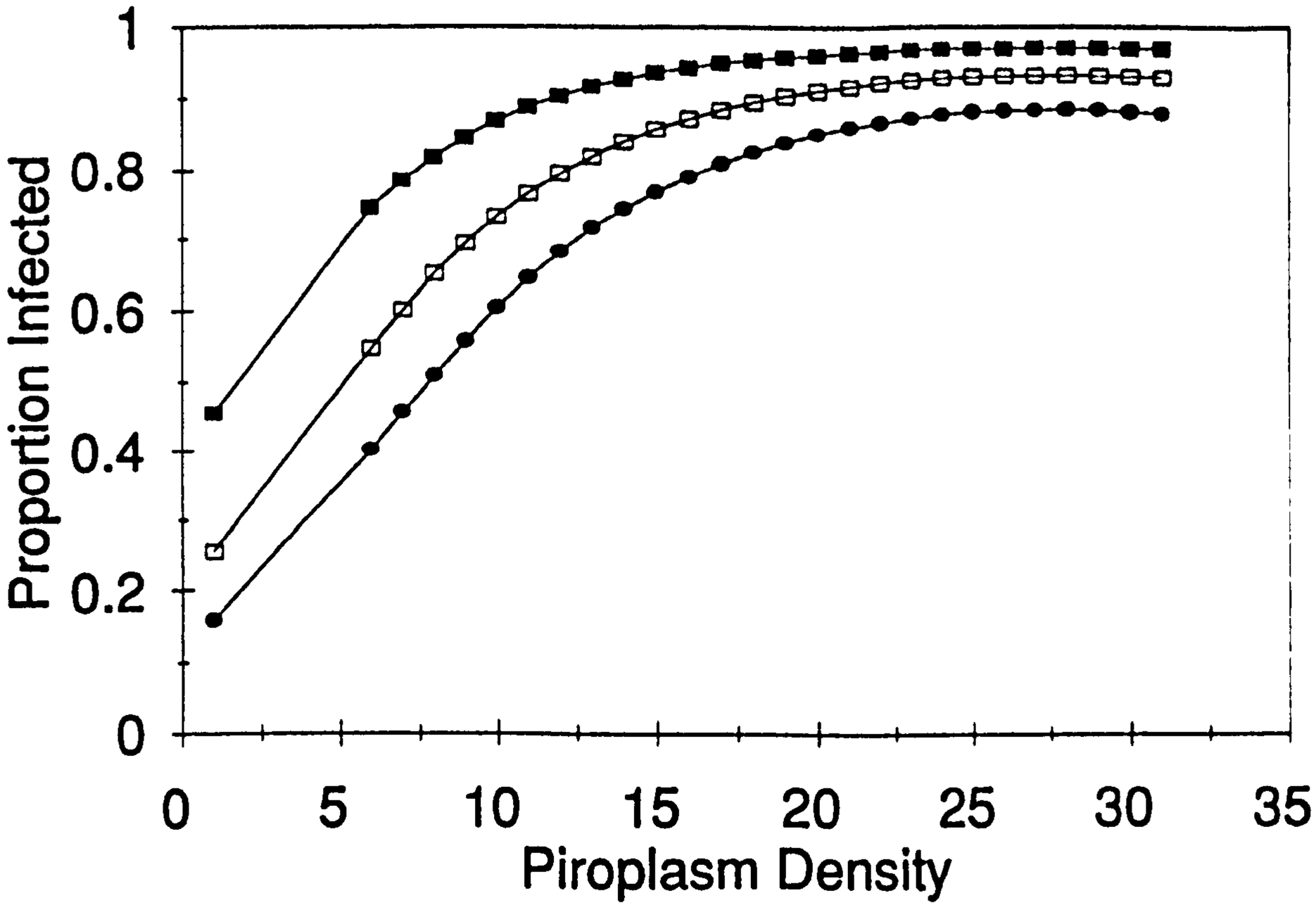
Table 6.2 Sequential combinations of pairs of adjacent batches and the resulting differences in the log likelihood from the "Full batch model". All values at 1 degree of freedom, χ^2 for significance at $\alpha = 0.05$.

Batch dummy variables	Log likelihood differences	P values
B1+B2 (vs B3, B4, B5)	39.992	<0.01
B2+B3 (vs B1, B4, B5)	32.503	<0.01
B3+B4 (vs B1, B2, B5)	2.140	>0.10
B4+B5 (vs B1, B2, B3)	5.697	<0.05
B3+B5 (vs B1, B2, B4)	0.856	>0.30

Effect of bovine host: The difference between the model containing the 3 dummy variables for the effect of the 4 steers and the one containing only the instar/gender variable was not significant ($P > 0.05$) (Table 6.1). However the dummy variables for bovine host effects remained forced into the model, as a confounding factor.

Effect of piroplasm density: Terms of piroplasm density, both linear and non-linear (quadratic and cubic transformations), were included into the model to evaluate their contribution to increasing the overall fit of the model (Table 6.1). Addition of the linear term of piroplasm density to the model containing only instar/gender variables increased the model fit significantly ($P < 0.01$). Addition of the quadratic piroplasm term further raised the log likelihood by 61.834 ($P < 0.01$). There was no significant difference when the cubic piroplasm transformation was added to the model ($P > 0.05$). Figure 6.4 shows the results of the best fitting model in equation (3) using the parameter values given in Table 6.3 for bovine host 1 and 4.

Effect of days post-infection: Inclusion into the basic model of the linear term of the continuous variable "days post-infection" statistically improved the model fit ($P < 0.001$). When the quadratic term of the variable was also included into the model, it produced a marginally better fit (-2log likelihood = 396.776, d.f. = 4) than the model containing the B2 + BP5 reparameterized batch dummy variables (-2log likelihood = 393.651, d.f. = 4). These two models were not nested hence could not be compared directly. The minimal difference between the two models is expected as the batch variable was effectively a surrogate measure of "days post-infection". Addition of the cubic term did not improve the model significantly ($P > 0.05$). Figure 6.5 shows the results of the best fitting model in equation (3) using the parameter values in Table 6.3.



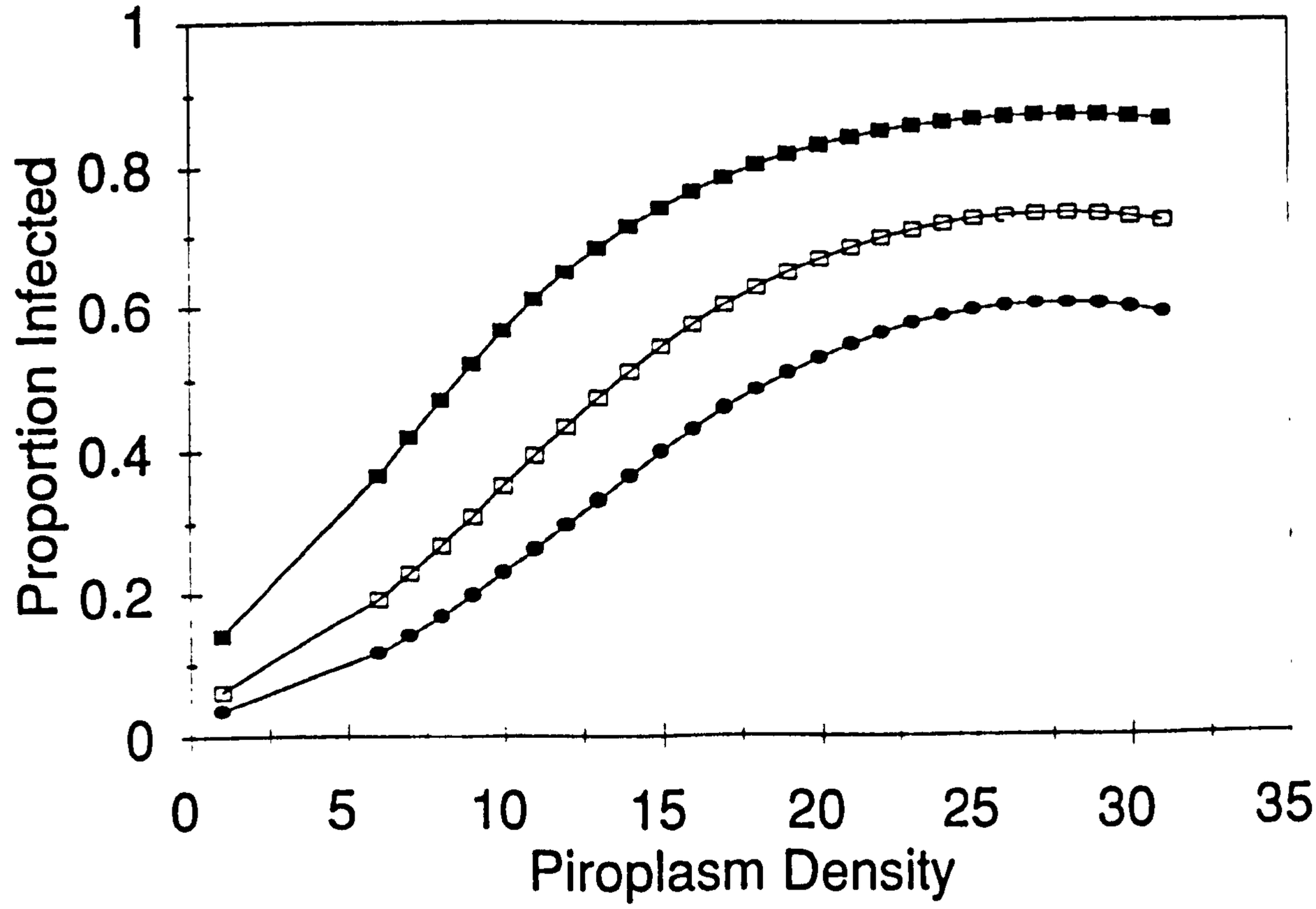


Figure 6.4. Proportion of infected *Rhipicephalus appendiculatus* nymphae (●) and adult males (□) and females (■) plotted against bovine host piroplasm density. The lines show the results of the model in equation (3) using the parameter values given in Table 6.3 for bovine host 1 (top) and 4 (bottom).

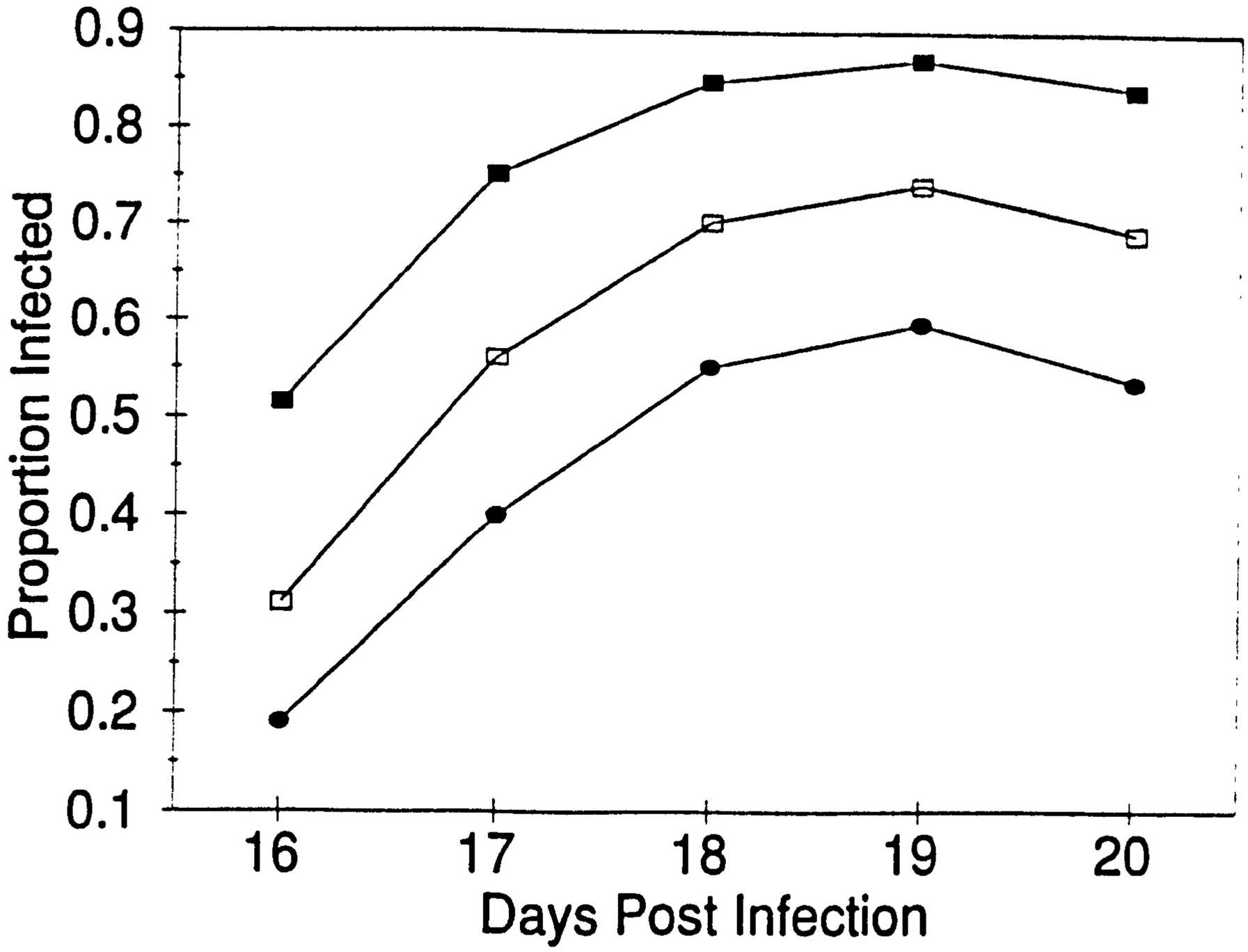


Figure 6.5. Expected proportion of infected *Rhipicephalus appendiculatus* nymphae (●) and adult males (□) and females (■) after feeding to repletion on a bovine host on days 16 to 20 after sporozoite stabilate inoculation. The lines on the graph are results of the model in equation (3) using the parameter estimates for Model 1 in Table 6.3.

Table 6.3. Parameter estimates of the best fitting models for estimating the proportion of infected tick vectors and their abundance of infection.

		Variable	Parameter estimate
Proportions	Model 1	Intercept	-76.6498
		Male	0.6541
		Female	1.5094
		Days post-infection (linear)	8.1380
		Days post-infection (quadratic)	-0.2147
	Model 2	Intercept	-3.5821
		Male	0.5929
		Female	1.4842
		Piroplasm density (linear)	0.2857
		Piroplasm density (quadratic)	0.0052
		Bovine host 2	0.5869
		Bovine host 3	0.9817
		Bovine host 4	1.6280
Abundance	Model 3	Intercept	-38.6900
		Instar 2	1.6890
		Instar 3	2.4420
		Days post-infection (linear)	4.2470
		Days post-infection (quadratic)	-0.1151
		Bovine host 2	0.1215
		Bovine host 3	0.3376
		Bovine host 4	0.9518
		Piroplasm density (linear)	0.0933
		Piroplasm density (quadratic)	-0.0012

Abundance of infection

Datafile1 (excluding piroplasm values): The independent variables were bovine host, tick instar/sex, and days post infection and the dependent variable was number of acini infected.

A histogram for the dependent variable showed a markedly skewed distribution (Figure 6.1). The huge difference between the mean (26.74) and the variance (4251) implied that the distribution was unlikely to be truly Poisson. This was confirmed when the minimal model, with the intercept only, was fitted. The scaled deviance: degrees of freedom ratio was very large (191,307:2,399) compared to the 1:1 of a perfectly fitting Poisson distribution. However the various parameters were fitted first before the amount of residual deviance could be accurately assessed. All the independent variables were significant to the model fit (Table 6.4) hence the estimates for the model were calculated. However the final fit of the model was poor as the ratio of the scaled deviance to the residual degrees of freedom was very large (52.42). In addition the Q-plot of the standardized residuals was different from the expected straight line and its correlation coefficient was relatively low (0.8178). Likewise, Pearson's residuals plotted against the fitted values of the model showed that the model did not fit the data well. The residuals, apart from being large (between -10.00 and 70.00), were predominantly positive values. Plots of the residuals against the independent variables showed a systematic trend of increasing variance of standardized residuals over the three instars, suggesting that there was a systematic trend for which the model was not accounting for well. No obvious trends were noted for the other independent variables.

Table 6.4. Values of the changes in scaled deviance and degrees of freedom from those of the minimal model after sequential addition of parameters. The scale parameter values were unity for all models. OV5(Datafile1) is Datafile1 after adjustment for overdispersion using the negative binomial analogue. Log Datafile1 was the log transformation on the dependent variable (number of infected acini). s.d. = scaled deviance; d.f. = degrees of freedom; P = probability value.

Model parameters	Datafile1			OV5(Datafile1)			Log Datafile1			OV5(Log Datafile1)		
	s.d.	d.f.	P	s.d.	d.f.	P	s.d.	d.f.	P	s.d.	d.f.	P
+Instar	53523	2	<0.01	2465	2	<0.01	754.1	2	<0.01	554.0	2	<0.01
+Instar,Days	5794	1	<0.01	267	1	<0.01	274.2	1	<0.01	201.4	1	<0.01
+Instar,Days, Day^2	4290	1	<0.01	198	1	<0.01	166.2	1	<0.01	122.1	1	<0.01
+Instar,Days, Day^2,Cow	2314	3	<0.01	107	3	<0.01	19.1	3	<0.01	14.0	3	<0.01

Even under the conservative OV4 approach, the parameters remained significant to the fit of the model ($P < 0.01$).

When the OV5 macro was used, the final model had a residual scaled deviance much closer to its degrees of freedom compared to the first model (ratio of 107:3 respectively). This indicated that the fit was superior, hence the negative binomial distributional assumptions were more valid than those of the Poisson distribution before adjustment. Furthermore, the contribution of each variable to the fit of the model was still statistically significant ($P < 0.01$) based on the values of the residual scaled deviance (Table 6.4). Under this adjustment, the standardized residuals, although still exhibiting some bias towards positive values (between -0.500 and 8.000), were not as large as those under the Poisson distribution and the systematic increase in variance of the standardized residuals over the instars disappeared.

In the final approach, the log-transformed variable exhibited a histogram and a mean/variance ratio (1.616/3.145) suggestive of a Poisson distribution. The Q-plot was nearly a straight line with slope = 0.9879. The standardized residuals gave a better fit than for all the previous models (between -2.000 and 4.000) and they were randomly distributed. There were no systematic increasing patterns in variance of the standardized residuals for all the variables. The variables were all still significant to the model fit even after the OV4 macro was fitted to account for overdispersion ($P < 0.01$). The fit was not markedly improved by accounting for potential overdispersion. When the negative binomial analogue model was fitted to the transformed model, the improvement was not significant, neither was any variable made insignificant by use of the negative binomial analogue (Table 6.4).

Datafile2 (including recorded piroplasm values): With the exception of two additional variables, linear and quadratic terms of piroplasm density, the method and approach to analysis of this data set was identical to that used for Datafile1.

The histogram of the data was again markedly skewed. The difference between the mean and variance was large (26.87 and 4303 respectively), implying that the distributional assumption was erroneous. The scaled deviance:degrees of freedom ratio of the minimal model was very large (182,992:2,279). All the independent variables were significant to the model fit ($P < 0.05$) even though the final fit of the model was poor (ratio of scaled deviance to residual degrees of freedom = 52.74). Similarly, the Q-plot did not produce a straight line and the correlation coefficient was 0.8188. The standardized residuals were predominantly positive values and ranged between -12.00 and 68.00. Plots of the standardized residuals against independent variables showed a systematic increasing trend over the three instars. The other variables did not show any obvious trend. The parameters remained significant to the model fit ($P < 0.01$) even after the poor fit of the model was attributed to overdispersion using the OV4 macro.

Values of the changes in scaled deviance and degrees of freedom from the minimal models after sequential addition of the variables when the negative binomial analogue was used are shown in Table 6.5. All the independent variables remained significant to the model fit ($P < 0.01$). The standardized residuals were predominantly positive values and ranged between -0.800 and 7.200 even though most of the values were below 4.000. There was no systematic increasing trend over the instars when the standardized residuals were plotted against the independent variables. The other variables did not show any obvious trend either. The results of the best fitting model

in equation (4) using the parameter values in Table 6.3 (Model 3) are shown in Figure 6.6.

The log transformed variable had a mean:variance ratio of 1.614 : 3.154 and a Q-plot with a correlation coefficient of 0.9876. The changes in scaled deviance values and degrees of freedom are given in Table 6.4. All the independent variables were significant to the model fit ($P < 0.01$). The values of the standardized residuals plotted against fitted values were between -2.000 and 4.400. The scaled deviance values obtained after fitting a negative binomial analogue model (OV5) are given in (Table 6.5).

Datafile3 (including an imputed piroplasm value): Results for the analysis of Datafile3 are given in (Table 6.6). The results were all similar to those obtained for Datafile2. All the independent variables remained significant to the model fit ($P < 0.01$) in all instances.

Table 6.5. Values of the changes in scaled deviance and degrees of freedom from those of the minimal model after sequential addition of parameters. The scale parameter values were unity for all models. OV5(Datafile2) is Datafile2 after adjustment for overdispersion using the negative binomial analogue. Log Datafile2 was the log transformation on the dependent variable (number of infected acini). s.d. = scaled deviance; d.f. = degrees of freedom; P = probability value.

Model parameters	Datafile2			OV5(Datafile2)			Log Datafile2			OV5(Log Datafile2)		
	s.d.	d.f.	P	s.d.	d.f.	P	s.d.	d.f.	P	s.d.	d.f.	P
+Instar	50800	2	<0.01	2511	2	<0.01	698.6	2	<0.01	509.2	2	<0.01
+Instar,Days	6695	1	<0.01	331	1	<0.01	301.1	1	<0.01	219.5	1	<0.01
+Instar,Days, Day^2	3548	1	<0.01	175	1	<0.01	142.3	1	<0.01	103.7	1	<0.01
+Instar,Days, Day^2,Cow	2131	3	<0.01	105	3	<0.01	15.9	3	<0.01	11.6	3	<0.01
+Instar,Days, Day^2,Cow,Piro	335	1	<0.01	17	1	<0.01	0.9	1	<0.01	0.7	1	>0.05
+Instar,Days, Day^2,Cow,Piro^2	96	1	<0.01	5	1	<0.01	5.1	1	<0.01	3.7	1	=0.05

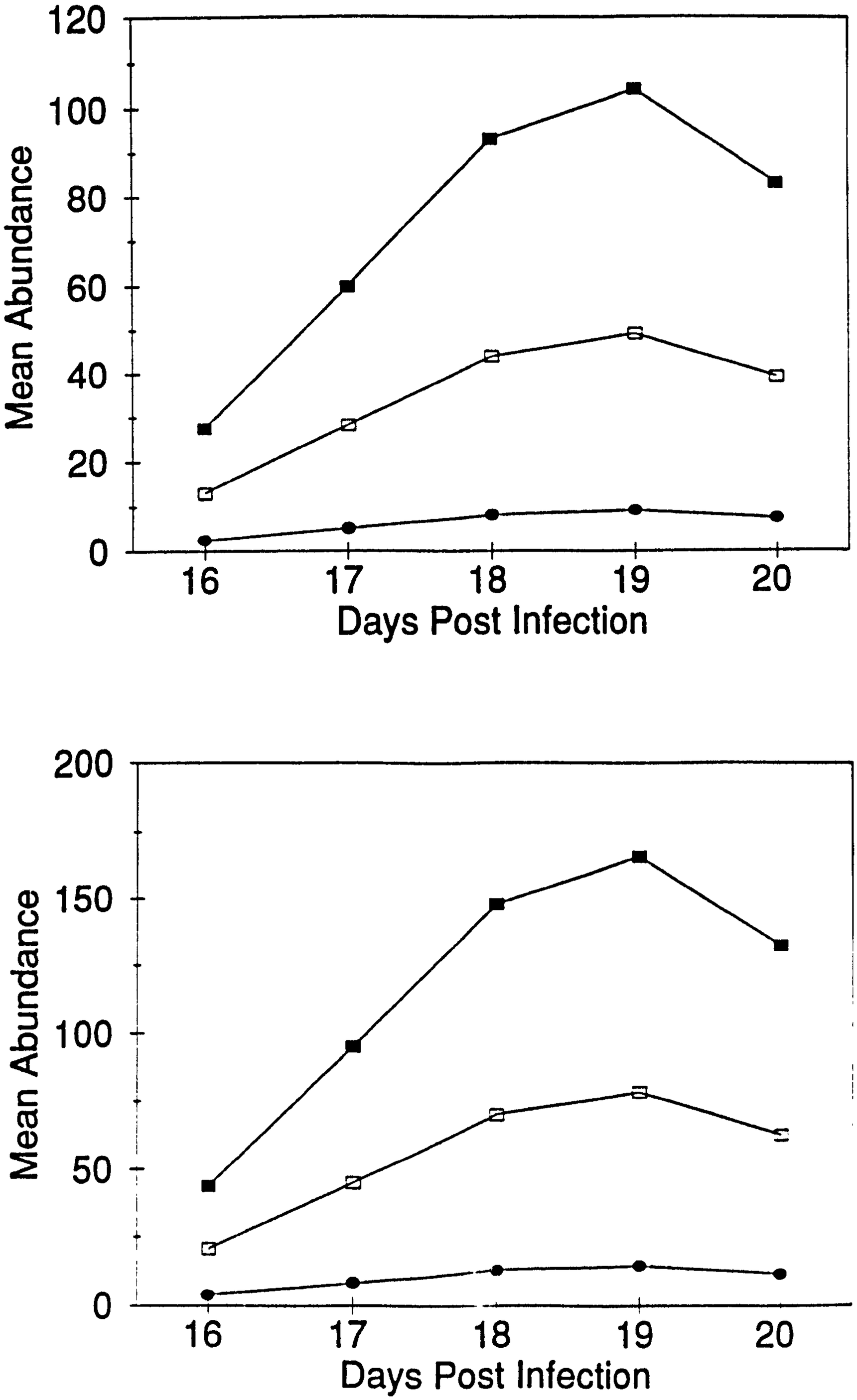


Figure 6.6. Expected mean abundance in *Rhipicephalus appendiculatus* nymphae (●) and adult males (□) and females (■) after feeding to repletion on respective bovine hosts (host 1 top and host 4 bottom) on days 16 to 20 after sporozoite stabilate inoculation. The lines on the graphs are results of the model in equation (4) using the parameter estimates for Model 3 in Table 6.3.

Table 6.6. Values of the changes in scaled deviance and degrees of freedom from those of the minimal model after sequential addition of parameters. The scale parameter values were unity for all models. OV5(Datafile3) is Datafile3 after adjustment for overdispersion using the negative binomial analogue. Log Datafile3 was the log transformation on the dependent variable (number of infected acini). s.d. = scaled deviance; d.f. = degrees of freedom; P = probability value.

Model parameters	Datafile3			OV5(Datafile3)			Log Datafile3			OV5(Log Datafile3)		
	s.d.	d.f.	P	s.d.	d.f.	P	s.d.	d.f.	P	s.d.	d.f.	P
+Instar	53523	2	<0.01	2707	2	<0.01	754.1	2	<0.01	555.9	2	<0.01
+Instar,Days	5794	1	<0.01	293	1	<0.01	274.2	1	<0.01	202.1	1	<0.01
+Instar,Days, Day^2	4290	1	<0.01	217	1	<0.01	166.2	1	<0.01	122.5	1	<0.01
+Instar,Days, Day^2,Cow	2314	3	<0.01	117	3	<0.01	19.1	3	<0.01	14.0	3	<0.01
+Instar,Days, Day^2,Cow,Piro	339	1	<0.01	17	1	<0.01	0.9	1	>0.05	0.7	1	>0.05
+Instar,Days, Day^2,Cow,$Piro^2$	77	1	<0.01	4	1	=0.05	3.7	1	=0.05	2.8	1	=0.05

6.3.2 Vector Competence

Prevalence of infection

Using logistic regression, all the explanatory variables (tick stock, parasite stock, tick instar/gender, bovine host effects, batch of origin and day of repletion after infection of the bovine host) had a significant effect on the fit of the model ($P < 0.05$) to the dependent variable (prevalence of infection). The effect of the linear and quadratic component of days post-infection provided as good a fit as batch effect to the model but for one less degree of freedom.

The binomial distributional assumptions appeared adequate when tested using the Q-plot (which resulted in a nearly straight line with a correlation coefficient of 0.9986). However, when the standardized residuals were plotted against the fitted values of the model, there were several large residuals (33 values out of 140 were outside the range of -3 to +3) even though their pattern was acceptable as it had no trend. This indicated that the model was not describing all the variation observed, which could be due to an unobserved explanatory variable or variables.

Overdispersion within the data was accounted for by using the OV1, OV2 and OV3 macros (Section 6.2.2). While adjustment using all the three macros improved the model fit significantly ($P < 0.01$), all the variables remained significant to the overall fit of the model except the quadratic component of days post-infection of the bovine host. Thus, the terms of the final model for the prevalence of infection in the different tick stocks were, parasite stock, tick stock, tick gender, cow and the linear component of days post-infection of the bovine host.

Abundance of infection

The mean:variance ratio was 42.88:2103. The initial overall fit of the model under Poisson distributional assumptions before adjustment was very poor as demonstrated by the large values of the residual scaled deviance compared to the residual degrees of freedom (majority of the values were outside the range of -3 to 3, the highest being 28). Likewise, the poor fit of the model was demonstrated by the plots of the standardized residuals which had very large values and the Q-plot, which did not exhibit a straight line and whose correlation coefficient was 0.9358. The negative binomial distributional analogue macro (OV5) was thus used to account for overdispersion. The OV5 macro improved the fit of the model significantly ($P < 0.001$). However all the variables remained significant to the model fit except the quadratic component of days post-infection. The improved fit of this model was confirmed by the standardized residual plot which even though still exhibiting some large residuals, were fewer than under the unadjusted Poisson distribution model. The distribution of residuals over the explanatory variables did not show any trends that the model could be missing to account for adequately. Even under the conservative OV4 macro approach, the only variable that became insignificant to the model fit was the quadratic component of days post-infection.

As in the comparison of nymphal and adult transmission, any significant effect the bovine host factor had on the fit of the model was dependent on other variables. When included in the full model containing all the four variables (tick gender, tick stock, parasite stock and days post-infection), the bovine host factor was not significant ($P > 0.05$). However, when included in the model lacking days post-infection, the bovine host factor became significant to the fit of the model ($P < 0.01$). Thus, the variables included in the final model under the negative binomial

distributional analogue macro (OV5) were tick gender, tick stock, parasite stock, days post-infection in the bovine host.

6.3.3 Tick survival and decline of *T. parva* infections

Parasite survival

Under distributional assumptions which had not been adjusted for overdispersion, all the individual variables, including the quadratic and the cubic components of time were all significant to the fit of the model ($P < 0.01$). Thus the model at this stage contained level of infection, instar, conditions of tick exposure, the linear, quadratic and cubic components of time after tick exposure. The Q-plot of the distributional assumptions did not produce a straight line and the correlation coefficient was relatively low (0.8686) implying a poor fit of the model. Even though the standardized residuals plotted against the fitted values did not show any particular pattern, some values were relative large (10.00 to -8.00). The standardized residuals plotted against the independent variables did not show any particular trend except for the levels of infection which increased steadily. This meant that the model was not fitting the data well. Analysis was thus repeated using the negative binomial analogue (OV5). Again all the variables were significant to the fit of the model ($P < 0.01$). The Q-plot of the standardized residuals produced a line closer to being straight than that in the model whose distributional assumptions were not adjusted. The standardized residuals plotted against the fitted values did not show any particular pattern and the values were smaller in magnitude (3.90 to -1.50). Apart from a systematic decrease across the instars, all the other variables did not show any pattern when the standardized residuals were plotted against the independent variables. This was an improvement to the first model. Thus in the final model

containing instars, levels of infection, conditions of tick exposure and the linear, quadratic and cubic components of time after tick exposure to the various conditions, the negative binomial analogue model, being of better fit, was used.

Tick survival

All the individual variables were significant to the fit of the model ($P < 0.01$) under the unadjusted distributional assumptions.

The Q-plot of the distributional assumptions produced a nearly straight line with a relatively high correlation coefficient (0.9843). When the standardized residuals were plotted against the fitted values, the limits were 4.80 and -16.80. The pattern was unusual, as anticipated, owing to the paucity of data between the extremes of all dead compared to all those alive. This implied that the model was not fitting the data adequately. The standardized residuals plotted against the independent variables did not show any particular trends across the variables.

Improvement of the model fit was attempted using the OV1, OV2 and the (OV3) macros. All the variables remained significant ($P < 0.05$) to the model fit under the three macros. The ranges of standardized residuals plotted against the fitted values were all lower in value compared to that in the unmodified model and did not show any particular pattern. The standardized residuals plotted against the covariates did not follow any particular pattern. This implied that the distributional assumptions of the three macros were more correct. However, the OV3 macro model was marginally better than the other two. The final model was thus the OV3 model containing instars, levels of infection, conditions of tick exposure and the linear, quadratic and cubic components of time after tick exposure to the various conditions.

6.4 DISCUSSION

As in most parasitic populations, *Theileria parva* demonstrated an overdispersed distribution within its tick vector. This distribution has been reported only once before (Büscher & Otim, 1986). Overdispersion could be caused by tick factors (such as instar/gender, differences in susceptibility due to genetic variation), parasitic factors (for example, genetic variation and success of syngamy) environmental and random effects (for example, chance death of parasites). It was no surprise therefore that the negative binomial analogue model provided the best fit out of those examined. The negative binomial probability model has been shown to be a good descriptor of a wide variety of overdispersed parasite population distributions (Anderson & May, 1992; Medley *et al.*, 1993a; Billingsley, Medley, Charlwood & Sinden, 1994).

There were fewer highly infected nymphal than adult ticks (Figure 6.3). One explanation is that this could be due to the reduced numbers of type III acini available for infection in nymphal ticks (Table 3.4). The differences in type III acini between nymphal, male and female ticks is also a possible explanation for differences in prevalence and intensity of infection between the instars and, or gender, infection levels increasing with the number of type III acini. When all the ticks that fed to repletion over 5 consecutive days on four cattle were considered, the basic pattern of the frequency plot remained the same even though it has been shown that the mean of the distribution of the parasite in the tick changes with days post-infection (Section 6.3). This implies that causes of overdispersion were constant through the infectious period examined (day 16 to 20 post-infection of the bovine host) and across the explanatory variables examined. It is likely that there are many

important factors involved in determining infection levels in ticks. Initially, variations in the blood meal, for example, size and composition, could be responsible for the variations in the number of ingested parasites. The blood meal is of obvious importance because it determines the initial amount of piroplasms that will be available for development in the tick. By the time ticks are replete, they have already concentrated the blood meal to some degree such that it is more than that indicated purely by the weight of the engorged tick. However, it is not yet clear what proportion of piroplasms form gametes in the tick gut, but it is speculated that it is low, that is from 0 to 1/100. The number of parasites ingested are however not directly proportional to the number of parasites that eventually develop due to a number of hazards that the parasite is exposed to in the tick. Walker (1990) has postulated that the hazards include the proteolytic digestive enzymes secreted into the gut during digestion of a blood meal, followed by ingestion by gut phagocytic cells. Young and Leitch (1981) also demonstrated that extreme temperatures are detrimental to the development of *T. parva* in *R. appendiculatus* nymphae. The number of type III acini available for infection becomes important after development of surviving parasites in the tick gut. The effect of the hazardous factors discussed above on parasite transmission to ticks can be assessed by calculating the probability of infection per acinus within instars/gender. These probabilities being unequal suggests that other tick factors, outlined above, play a pivotal role in determining vector competence. This result is shown in Figure 6.4, where it can be seen that if a nymphal tick is infected, a much higher proportion of its acini are infected than either male or female ticks. The causes and role of this pattern have yet to be fully explained.

One notable feature in the comparison of nymphal transmission to that of the adult and vector competence was the effect of the bovine host factors on parasite development in the tick. These effects became significant only when days post-infection or batch was not considered. Further, the bovine host effect became significant ($P < 0.01$) when piroplasm density was incorporated. The implications are two-fold. First, piroplasm parasitaemia density on its own cannot be used to predict prevalence of infection in ticks and must be complemented with other host-related variables. This may explain why Büscher and Tangus (1986) found no significant correlation between prevalence and mean intensity to piroplasm parasitaemia. Use of additional variables to predict tick infection levels is supported by the fact that nymphal ticks that feed to repletion on cattle during the rising phase of piroplasm parasitaemia develop higher infections than those feeding to repletion before or after this phase (Young *et al.*, in preparation a).

Secondly, it seems that there is an overriding factor in "days post-infection" that determines the prevalence of infection in ticks irrespective of the individual bovine host the ticks feed on. Based on the second implication above, it is possible that the age of the piroplasm could be an important factor in determining infection levels developing in ticks or that piroplasms themselves are not infectious to ticks. These results are consistent with another parasite stage (or possibly a subpopulation of total piroplasms), the density of which is correlated to total piroplasm density, being infectious to ticks. Piroplasm density is very low in carrier animals where parasitological techniques frequently fail to detect parasites in animals that are infectious to ticks. This further supports the supposition that piroplasm density is not an adequate measure of cattle infectiousness to ticks. Given the epidemiological

importance of measurement of infectiousness in cattle, this area deserves further investigation.

Parasite survival being longer in lowly infected ticks is important because *T. parva* infection levels in ticks in the field are usually very low (about 2 infected acini per female tick). This would thus allow for longer survival periods of the parasites. Unlike mosquitoes which seek their hosts actively over a relatively large area, movement of ticks is mainly restricted vertically up and down vegetation. Thus long survival periods are crucial to the parasite. It was also no surprise that the parasite survived best under field conditions. One problem that remains is trying to reproduce in the laboratory the ambient factors in the field that determine parasite survival.

In terms of analysis of abundance data, the negative binomial analogue model provided a much better fit compared to the Poisson distribution in all cases. Even though the log transformation on the number of acini provided a very good fit for the Poisson distribution, the use of the negative binomial model is worthwhile to check the robustness of parameter estimates in the face of overdispersion. However, the distributional assumptions with log transformed data are easier to manage, and given that the overall fit is better, this would suggest that log transformation is the most suitable method of analysis for future research on acini infection intensity.

This chapter of analysis has explained some of the primary causes of patterns of infection and survival. However the data are deserving of further analyses. In particular, the measurement of survival of *T. parva* and the tick hosts should be examined in greater depth to test more rigorously for the effect of density-

dependence. Medley *et al.* (1993b) and Billingsley *et al.* (1994) have shown that prevalence-intensity relationships in the malaria-mosquito system can be useful in describing the distribution of parasites. Preliminary analysis of the data in this thesis (not shown) and other data (Medley, personal communication) suggest that similar relationships exist for *T. parva* in *R. appendiculatus*. Throughout the analyses presented, the overdispersed distribution of *T. parva* in ticks played an important role and had to be carefully considered. The causes and consequences of this pattern are likely to be fruitful areas of future research.

CHAPTER 7

GENERAL DISCUSSION AND SUGGESTIONS FOR FURTHER STUDIES

Our knowledge of the life cycle of *Theileria parva* within its tick vector, *Rhipicephalus appendiculatus* and its mammalian hosts has increased tremendously over the past few years. This knowledge is very important in the understanding of the transmission dynamics of the parasite. Unfortunately, there still remain aspects of the biology of the parasite that contribute to its population dynamics that still need to be determined.

The dynamics of theilerial infections in the mammalian and tick hosts are important in the transmission of *T. parva* parasite which in turn drives the epidemiology of theileriosis. The two important interphases in the transmission dynamics of *T. parva* whereby the tick acquires infection from an infected mammalian host then transmits it (the infection) to a mammalian host are controlled by numerous factors. This study examined some of the factors affecting the population dynamics of *T. parva* in ticks that to date are still poorly understood. These were: comparative instar transmission, source of tick infection, vector competence and survival of the parasite and the infected tick vector under various climatic conditions. Values from these parameters can be used in developing simple population dynamics models for *T. parva* infections in ticks.

Previous work on the development of *Theileria* parasites within tick salivary glands of the tick vectors has been carried out mostly in the adult instar with that in the nymphal instar receiving scant attention. In view of the fact that more larval and nymphal ticks feed on cattle compared to adult ticks, and that larval/nymphal transmission cycle of *T. parva* is shorter than in the nymphal/adult cycle, it is important that development of *Theileria* in the nymphal instar should be given more attention than it has been given in the past.

Even though the male tick may not be as efficient as the female tick in transmitting the parasite due to the reduced numbers of infected acini and its erratic feeding pattern, there is another aspect that should be considered. As the female ticks drop replete from a bovine host, the male ticks still remain on the host for up to six or so weeks. This results in approximately three times more males remaining on the bovine host. This implies that male tick could transmit more parasites than the female ticks. Secondly, even though male ticks detach and reattach regularly to mate, it is possible that they can transmit the parasite rapidly after attachment, as was demonstrated in Ochanda *et al.* (1988), hence may still be efficient transmitters.

It seems that infections established in ticks feeding to repletion on carrier cattle vary considerably and this apparently depends on the stock of the parasite and the tick instar. The efficiency of *T. parva* stocks to induce a carrier state in cattle and buffalo vary from the least efficient *T. parva* Muguga, which is yet to be demonstrated to produce a carrier state, to the most efficient buffalo-derived stocks that are capable of inducing a 100% infection rate in adult ticks. Even though ticks that feed to repletion on cattle undergoing acute infections develop higher infection rates than those feeding on carrier cattle, the former infect ticks over a very short period (a few days) compared to carrier cattle that may remain infective to ticks for a number of years. This means that carrier cattle are more important in maintaining the parasite than cattle undergoing acute infections (Medley *et al.*, 1993).

In statistical analyses throughout all the experiments piroplasm density alone appeared to play an insignificant role in determining resultant infection levels in ticks. Even though cattle sample sizes were small, it is safe to conclude that

piroplasm density appeared to play an insignificant role in determining infection levels in ticks. The scatter of the values is so wide that even an increase in sample sizes would still not be enough to provide piroplasm density values that can on their own predict accurately infection levels developing in ticks.

The principles of development of *T. parva* in larval/nymphal and nymphal/adult instars have been laid down in this study. It must be remembered however that the number of salivary gland acini infected in the instars was used as the indicator of transmission. What remains now is to establish the comparative transmission by the two instars to find out if *T. parva* infections induced by nymphal ticks are likely to be less acute than those induced by adult ticks. This could be done either by tick application to susceptible cattle in culture or titration. In the former method, a balanced challenge of *T. parva* to susceptible cattle by an equal number of nymphal or adult ticks with similar but relatively low infections could be provided.

Tick survival was basically the same under either laboratory or field conditions. However parasite survival was different. It seems that there are conditions in the field that were not being duplicated in the laboratory. This cannot be the ambient moisture content because this did not have an effect on the different categories of infection levels in ticks. Two possible conclusions can be drawn from this: 1) Some unknown field factor or factors determine survival of the parasite. 2) The parasite density affects the parasite's survival in the tick salivary glands. Again, particularly for laboratory maintenance of *T. parva*, these factors should be studied further.

Basically, it seems that there are no significant differences in the numbers of sporozoites in the nymphal or adult tick salivary gland acinus even though it is

possible that the nymphal tick may have marginally less sporozoites in each acinus. However the eventual number of sporozoites would depend on the stage of parasite development, that is the number of divisions the parasite has undergone in the salivary gland during sporogony. The number of sporozoites will have to be determined more accurately in the different instars.

In this study only a few variables were chosen for investigation. Obviously there could be others that are additionally responsible for infection levels developing in ticks. A stricter control of the variables may be necessary to pinpoint and analyse the effects of individual variables more accurately. In particular, finding a measure of mammalian host infectiousness more accurate than piroplasm density is important.

Unlike in *Plasmodium* species, the *T. parva* gametocyte has not been unequivocally demonstrated. However, it is believed that they are the first stage division of ring form piroplasms. The kinete and the salivary gland stages have been shown to be haploid. A sexual cycle has also been demonstrated by Morzaria, Young, Bishop, Young, Dolan & Mehlhorn (1992). Recombinant studies that have been done also shows the great prospects of differences in tick stock transmission.

The transmission dynamics of *T. parva* are greatly influenced by the population dynamics of *R. appendiculatus*. A major feature of the tick population dynamics is the presence of behavioural diapause in adult ticks in central and southern Africa which results in seasonal infestation by tick instars and seasonal transmission of *T. parva*. In eastern Africa, in the absence of behavioural diapause, infestation by tick instars and *T. parva* transmission can occur throughout the year. The effect of diapause on the transmission of *T. parva* is being investigated.

The demonstrated differences in tick stock transmission has helped to explain a lot of phenomena which could not be done before. For example the observed epidemiological differences of *T. parva* in southern African region which is quite different from that observed in eastern African regions. This is an area of tremendous potential for further research. It seems that the tick selects the parasite rather than the parasite selecting the tick. However there is obviously a great deal of selection pressure on the ticks due to the two weekly dipping regime for tick and tick borne disease control strategy practised in general.

CHAPTER 8

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APPENDIX

PUBLICATIONS:

OCHANDA, H., YOUNG, A.S., MUTUGI, J.J., MUMO, J & OMWOYO, P.L. (1988). The effect of temperature on the rate of transmission of *Theileria parva parva* infection to cattle by its tick vector, *Rhipicephalus appendiculatus. Parasitology 97*, 239-45.

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PAPERS IN PREPARATION:

YOUNG, A.S., DOLAN, R.B., DOLAN, T.T., MWAKIMA, F.N., OCHANDA, H., MWAURA, S.N., NJIHIA, G.M. & MUTHONI, M.W. (submitted). Estimation of the heritability to infection with *Theileria parva* of *Rhipicephalus appendiculatus* ticks. *Parasitology.*

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OCHANDA, H., YOUNG, A.S., MWAKIMA, F.N. & MEDLEY, G.F. (in preparation). Vector competence of rhipicephalid tick stocks from different parts of Africa for the transmission of *Theileria parva* stocks from Kenya and Zimbabwe.

OCHANDA, H., YOUNG, A.S., MEDLEY, G.F. & PERRY, B.D. (in preparation). Survival of *Theileria parva* in instars of its tick vector *Rhipicephalus appendiculatus* under different conditions.

YOUNG, A.S., MWAKIMA, F.N., OCHANDA, H., DOLAN, T.T., NORVAL, R.A.I., PERRY, B.D., BERKVENS, D.L. & PEGRAM, R.G. (in preparation). The importance of behavioural diapause of adult *Rhipicephalus appendiculatus* stocks in the population dynamics in eastern, central and southern Africa.

PRESENTATIONS:

OCHANDA, H., YOUNG, A.S., MEDLEY, G.F., SHAW, M.K. & PERRY, B.D. Efficiency of transmission of *Theileria parva* stocks by different instars of *Rhipicephalus appendiculatus.* Presentation No. 054 at the British Society for Parasitology Spring Meeting held at the University of Bath on 6th - 8th April, 1994.

ADDENDA

I) A description of other *Theileria* species is summarized in Table 1.1, page 9b

II) The following statement should appear in page 58 after "...mosquito salivary glands (Warbug & Miller, 1991)."

> "Indeed, Kamwendo, Ingram, Musisi, Trees and Molyneux (1993) have demonstrated, by surface lectin binding, consistent differences between the hoemocoelic surface carbohydrate composition of *R. appendiculatus* salivary gland acini types. These differences may correlate with the specific susceptibility of the type III acini to *T. parva* infection."

III) The legends for figures in Chapter 5 are as follows:

Figure 5.2. Survival of *Theileria parva* Muguga in the highly infected adult *Rhipicephalus appendiculatus* Muguga after exposure to different climatic conditions.

The ticks were assessed for salivary gland infections at monthly intervals. (● = 20°C, 85% RH; O = 24°C, 85% RH; ■ = 13-23°C (diurnal fluctuation), 85% RH; □ = 23-30°C (diurnal fluctuation), 85% RH; ▲ = quasi-natural climatic conditions).

Figure 5.3. Survival of *Theileria parva* Muguga in adult *Rhipicephalus appendiculatus* Muguga having medium and low infections after exposure to different climatic conditions.

Conditions of exposure were: 20°C, 85% RH (● = ticks of medium infections; ■ = ticks of low infections) or quasi-natural climatic conditions (O = ticks of medium infections; □ = ticks of low infections) and sampled at monthly intervals.

Figure 5.3. Survival of *Theileria parva* Muguga in adult *Rhipicephalus appendiculatus* Muguga having medium and low infections after exposure to different climatic conditions.

Conditions of exposure were: 20°C, 85% RH (● = ticks of medium infections; ■ = ticks of low infections) or quasi-natural climatic conditions (O = ticks of medium infections; □ = ticks of low infections) and sampled at monthly intervals.

Figure 5.5. Survival of adult *Rhipicephalus appendiculatus* Muguga highly infected with *Theileria parva* Muguga after exposure to different laboratory and quasi-natural conditions.

Surviving ticks were counted at monthly intervals. ● = 20°C, 85% RH; ○ = 24°C, 85% RH; ■ = 13-23°C (diurnal fluctuation), 85% RH; □ = 23-30°C (diurnal fluctuation), 85% RH; ▲ = quasi-natural climatic conditions.

Figure 5.6. Survival of adult *Rhipicephalus appendiculatus* Muguga having medium and low infections of *Theileria parva* Muguga after exposure to different climatic conditions.

Conditions of exposure were: 20°C, 85% RH (● = ticks of medium infections; ■ = ticks of low infections) or quasi-natural climatic conditions (○ = ticks of medium infections; □ = ticks of low infections) and surviving ticks counted at monthly intervals.

Figure 5.7. Survival of the highly and lowly infected nymphal *Rhipicephalus appendiculatus* Muguga after exposure to different climatic conditions.

Conditions of exposure were: 20°C, 85% RH (● = ticks of medium infections; ■ = ticks of low infections) or quasi-natural climatic conditions (○ = ticks of medium infections; □ = ticks of low infections) and surviving ticks counted at monthly intervals.

IV) The following publication should appear in the references:

KAMWENDO, S.P., INGRAM, G.A., MUSISI, F.L., TREES, A.J. & MOLYNEUX, D.H. (1993). Characteristics of tick, *Rhipicephalus appendiculatus*, glands distinguished by surface lectin binding. *Annals of Tropical Medicine and Parasitology 87,* 525-35.